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ANOTHER CONIDIAL SCLEROSPORA OF PHILIPPINE MAIZE

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Each year in the Philippine Islands the valuable maize crop suffers very severe losses from the destructive activities of downy mildew (*Sclerospora* spp.). While the writer was studying this disease during the past two years his attention was naturally directed to the question whether the widespread destruction of maize throughout the thousand-mile extent of these scattered islands was due in all cases to the same species of fungus. A comparative study of material collected from many parts of the provinces of Batangas, Laguna, and Rizal in the island of Luzon, where the disease is most serious and where it was studied most intimately, showed that in all cases the same causal fungus was involved. This species of downy mildew was described in an earlier paper (*12*)[1] as *Sclerospora philippinensis*. It was only natural to suspect that some of the abundant Philippine wild grasses related more or less closely to maize would be found to harbor this or other Sclerosporas. As on the widely distributed wild grass *Saccharum spontaneum* L. (Pl. 77, A) the oogonial stage of a Sclerospora had been very commonly encountered in great abundance, this grass was obviously an object of suspicion. In Luzon, however, despite extensive search, no conidial stage was seen on this host.

During a trip to the more southern Visayan Islands of Cebu, Bohol, and Leyte, in which maize is a crop of very great importance, the writer found that there, also, the maize plantings were suffering heavy losses from downy mildew. As no microscope was carried, no study of the causal organism was made at night during the period of conidium production. However, inasmuch as the symptoms and the general effect of the downy mildew were the same in these southern islands, the writer inferred that the causal organism was that which he had found so widely distributed on maize throughout the northern island of Luzon. Also the wild grasses of these southern islands were carefully examined as possible

[1] Reference is made by number (italic) to "Literature cited," p. 684.

hosts for downy mildew. After long search a clump of bugang grass (*Saccharum spontaneum*) heavily infected by a conidial Sclerospora was discovered by Mrs. Weston. Continued hunting brought the fungus to light on the same host in two other places, all three cases being encountered in the rugged interior uplands of Cebu (Pl. 76), which lie between Carcar and Barili. In the island of Leyte, also, this Sclerospora was again found on bugang grass on a hillside about three miles from Baybay. No other cases of downy mildew either on this or on other hosts were seen. Later, in a field of native sugar cane near Guadelupe cemetery outside the town of Cebu, a single clump of cane was found infected with the conidial stage of a Sclerospora.

The infected plants of *Saccharum spontaneum* and sugar cane were transplanted to Los Baños, Luzon, for further study (Pl. 77, B). There a comparison of living material taken from these plants during the optimum time of nocturnal conidiophore production showed that this downy mildew from the southern islands was different from that previously studied in Luzon. This discovery necessitated a revision of all available material in order to determine whether or not other forms had been previously overlooked under the assumption that the collections were all of the same form so commonly found in Luzon. Accordingly, living material from maize, teosinte, and sorghum from the college plots and from native fields in Batangas and Laguna provinces was compared with the living material from the plants of *Saccharum spontaneum* and sugar cane brought from Cebu. Dried, preserved, and mounted specimens from maize collected in various parts of Luzon were compared with similar specimens from maize obtained in various localities in Cebu, Bohol, and Leyte. This survey showed clearly that all the material so far encountered fell into one or the other of two distinct species—one, the form with shorter, broader conidia found on maize, etc., in Luzon and previously described as *Sclerospora philippinensis*, and the other, which will be called *Sclerospora spontanea*, characterized by longer, narrower conidia, and found on maize, bugang grass, and sugar cane in the Visayas. Once this point had been established, a comprehensive study was made of the two species to determine the resemblances and differences between them in morphological and physiological characteristics.

COMPARATIVE STUDY OF SCLEROSPORA PHILIPPINENSIS AND SCLEROSPORA SPONTANEA

FIELD CHARACTERISTICS

On maize, as observed in the field in the more southern islands and in Luzon, the two species are apparently identical in their destructiveness to the crop as a whole and also in their effect on the individual plants. It is possible that quantitative studies of essentially similar fields infected by the separate species would show some slight differences, but in general appearance there is no distinction whatever between the two.

PHYSIOLOGICAL CHARACTERISTICS

Several varieties of maize grown in sterile soil and under controlled conditions preventing contamination were infected with spores produced on the living plants of buganğ grass (*Saccharum spontaneum*) and sugar cane brought from Cebu. Parallel inoculations were made also with *Sclerospora philippinensis*. No difference was apparent either in symptoms or in the virulence of the resulting infection. Similar experiments with seedlings of cultivated wheat, Setaria, Pennisetum, and several species of wild grasses, including the very common aguinğay (*Rottboellia exaltata* L.), anias (*Andropogon sorghum* var. *halepense* L.), cogon (*Imperata cylindracea* L.), and tigbee (*Coix lachryma-jobi* L.), using the long, narrow conidia of the southern species, were as uniformly unsuccessful as they had been with *Sclerospora philippinensis* (12). Seedlings of teosinte (*Euchlaena luxurians* Schrad.) and the wild grasses, *Saccharum spontaneum* and *Miscanthus japonicus* (Thunb.) Anders., were successfully inoculated with both forms. No seeds of sugar cane were available for planting. Had there been, there is little doubt in the mind of the writer that infections in this case also could have been obtained. A more detailed account of these inoculation experiments will be given in a later paper. It should be said here, however, that the effect of the Sclerosporas varied with the different hosts, being most destructive on maize and least so on buganğ grass; but the characteristic production of conidiophores took place with uniform regularity at night on all (Pl. 78, B).

A comparative study of material of *Sclerospora spontanea* on these different hosts showed that the distinguishing morphological characteristics of the fungus had not been altered in any way. Moreover, even after transition from one host to another through several generations, the species remained constant and in no way approached *S. philippinensis*. In like manner, after inoculating various hosts and passing through several generations, *S. philippinensis* also was quite unchanged and showed no tendency to approach the long-spored form.

The writer considers it quite possible that an exact statistical study of large numbers of individuals infected by each of the two fungi would reveal some slight quantitative difference in the area bearing conidia, or in the rate of growth of hyphae through the host, or in some other aspect not at once apparent to an ordinary comparative examination. It should be noted here, however, that there is certainly no noticeable physiologic difference between the two in virulence, range of hosts, or general course of the resulting disease they produce.

MORPHOLOGICAL CHARACTERISTICS

Therefore, because the two forms differ morphologically rather than physiologically, they were carefully compared in order to determine whether the points of difference were sufficiently stable and well marked

to establish the long-spored form as a species distinct from *Sclerospora philippinensis*.

MYCELIUM.—In morphological characteristics, extent, and relation to the host tissue, the mycelium of the two fungi showed no distinctions sufficiently marked or unvarying to warrant their use as a basis of separation. However, the club-shaped hyphae (conidiophore initials) which grow out through the stomata and develop into conidiophores are different in the two forms, those of the long-spored Sclerospora being markedly longer, more slender, and more irregular.

CONIDIOPHORES.—In general appearance the conidiophores of the two Sclerosporas are noticeably dissimilar, those of the Visayan form being markedly longer, more slender, and more spreadingly branched than those of *Sclerospora philippinensis*. On analyzing this dissimilarity the details of difference discussed in the following paragraphs are apparent.

The basal cell of the Visayan Sclerospora is very long (Pl. 79, A, D, E, F, H), strikingly longer than that of *Sclerospora philippinensis*. The length (140 to 260 μ) is greater not only actually but also relatively, for even in the unusual cases when it is less conspicuously long (Pl. 79, G) the basal cell of the Visayan Sclerospora always exceeds or at least equals in length that part of the main axis extending from the terminal septum of the basal cell to the origin of the primary branches. In *S. philippinensis*, the basal cell is always shorter than this part of the main axis. Moreover, the basal cell of the Visayan Sclerospora is much more slender, usually 5 to 8 μ at its narrowest diameter, and much less knobbed or swollen at its base (Pl 79, A, D, E, F, H) than is the basal cell of *S. philippinensis*.

The main axis of the Visayan Sclerospora expands more abruptly above the basal cell and then constricts more distinctly (Pl. 79, A, D) just below the branches than in *Sclerospora philippinensis*. The greatest diameter (22 to 32 μ), which usually slightly exceeds that of *S. philippinensis*, is thus placed, not just below the branches (as in *S. philippinensis*), but some distance lower (Pl. 79, A, D, G, H).

The branches of the Visayan form generally are less constricted at their point of origin, are of more uniform diameter, and are straighter, less ascending, more spreading, and do not recurve, but stand out from the main axis more stiffly. They are characteristically longer and more slender, but, even if short and crowded, they stand out more stiffly than in *Sclerospora philippinensis*. Although varying considerably in both species, the number of conidia produced on conidiophores is approximately the same in *S. spontanea* and in *S. philippinensis*. In the former, 32 to 48 are commonly borne, although as many as 88 or as few as 12 may less frequently occur.

The sterigmata also are straighter, less recurved, and stand out more stiffly than in *Sclerospora philippinensis*, and, usually they are longer (about 13 μ). It should be noted, however, that the length varies with

the extent of the branch system, since in cases where this is reduced and the primary branches or even the main axis give rise directly to sterigmata, these sterigmata are much larger (Pl 79, B) than they are when arising from quaternary or tertiary branches as the ultimate structures of an elaborate system (Pl. 79, A).

As a result of such differences, the conidiophore top of the Visayan Sclerospora has a more spreading, expanded appearance; and the long axes of the branches, the sterigmata, and the conidia borne on them stand out from the main axis like rays of a partly opened fan. In *Sclerospora philippinensis*, on the contrary, the conidiophore top is more compact and less spreading, the axes of branches, sterigmata, and conidia being all approximately parallel to each other and to the main axis.

These differences in the conidiophores of the two fungi are, on the whole, relative rather than absolute and are influenced to some extent by such environmental conditions as the depth and persistence of the layer of dew in which they develop. Even these distinctions, however, could be used as more absolute and less relative criteria if a very large number of measurements of all parts of the conidiophores were made and assembled to give an adequate quantitative impression. Even from the qualitative rather than quantitative point of view, moreover, these differences, although relative, are constant and distinct, and it should be emphasized that they persist when the two fungi, developing under exactly parallel circumstances on sister plants of the same age, grown side by side under as nearly the same conditions of temperature, soil, dew deposition, etc., as it was possible to obtain, were compared by nightly examinations for several weeks.

CONIDIA.—Among the Peronosporaceae as a whole the characteristics of the conidia have been found to be the most valuable basis for distinguishing species. This applies equally well to these two Sclerosporas, since their conidia not only differ markedly and constantly in shape and size but also remain relatively unaffected by changes in environment and hosts.

In shape, the conidia of the Visayan Sclerospora are at once distinguished from those of *Sclerospora philippinensis*. They are not only much more elongate but much more slender as well, the length being frequently two or even three times the diameter. Consequently they range from very elongate ovoid and obovoidal bodies to long narrow, round-ended cylinders, but they are most commonly very elongately ellipsoid in shape. A clearer idea of these variations may be gained from Plate 79, I, J, K.

In such features as the rounded apex devoid of any papilla, the blunt base with its apiculus of attachment, the hyaline, granular content, and the thin wall, the conidia correspond to those of *Sclerospora*

philippinensis. As in the case of the latter species also, germination is invariably by the protrusion of one or more germ tubes (Pl. 79, I, J, K).

In size, the conidia of the Visayan Sclerospora are very variable. With respect to such widely varying bodies as the spores of this and other genera of Peronosporaceae, recent investigations have shown that it is no longer possible to delimit a species adequately by the extremes or averages of a few measurements. Rather, there is required the assembling and presentation in tables and graphs of a sufficiently large number of representative measurements to give a quantitative as well as a qualitative expression of the conidial characteristics of the species.

Accordingly, in order to obtain data adequate to identify the Visayan form and to furnish a basis for comparing it with others, 700 conidial measurements were made. These comprised measurement groups of 100 conidia from each of the two sugar-cane and the four *Saccharum spontaneum* plants from Cebu, and from one maize plant inoculated from the latter.

The conidia were taken from the leaves of the host at night during the optimum period of conidia production—from 2 to 4 a. m.—mounted in dew, and measured immediately.

Since, on examination, the seven measurement groups were found to agree in all essential particulars, they were combined into the total of 700. For the purposes of comparison, 700 measurements of *Sclerospora philippinensis* were secured in like manner.[1] Of these, 300 were new ones made of fresh conidia from teosinte and sorghum found infected in the college plots and from *Saccharum spontaneum* seedlings artificially inoculated from maize. All these groups were compared, found to agree, and grouped into the total of 700.

In making these measurements, care was taken to include every conidium in a marked area of the microscope field as the slide was moved along by the mechanical stage. Only those conidia obviously injured or those still attached to the conidiophores were excluded. The divisions of the eyepiece equaled approximately 1.8 μ, and, with the magnification used, it was possible to estimate with fair accuracy to one-third of a division, or to about 0.6 μ. Consequently, the measurements are exact to this extent—that is, the conidium recorded as 32 μ in length may as well be 31.4 μ or 32.6 μ instead of exactly 32 μ but not, in all probability, 31 or 33 μ. With a large number of spores such differences tend to equalize themselves. As a result, the measurements presented here may be considered as adequately representing the characteristics of the conidia of the species involved.

[1] The writer wishes to take this opportunity to call attention to an error in the tabulation of the previous spore measures of *Sclerospora philippinensis* (12, p. 110). In the table of length, the conidia measuring 41 to 42.9 μ should be 23 in number instead of 24.

The measurements are summed up in Table I and are presented in graphic form in figure 1. In addition, the biometric characteristics of the two species are given in Table II. In making the calculations, the directions and formulae of E. Davenport (3) and C. B. Davenport (2)

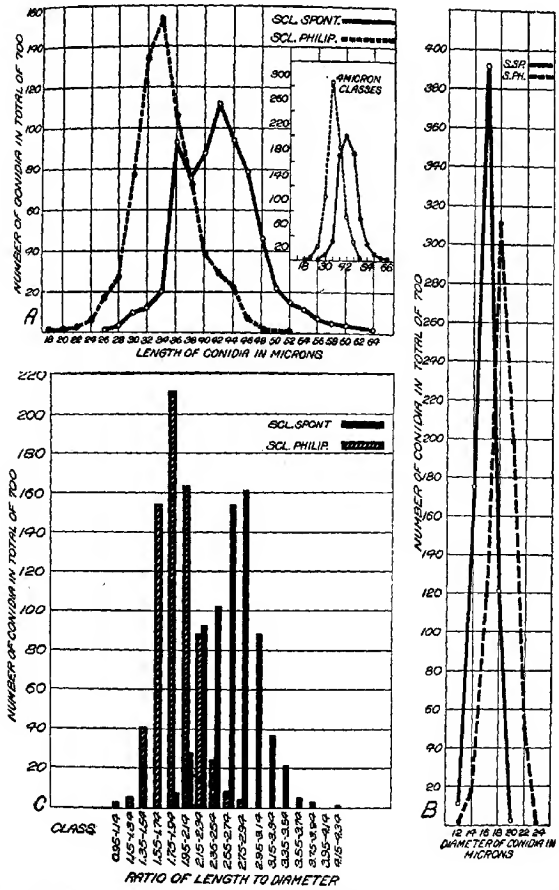

FIG. 1.—Comparison of the sizes of 700 conidia of *Sclerospora spontanea* with 700 conidia of *S. philippinensis*; *A*, variation of conidia in length; *B*, variation of conidia in diameter; *C*, ratios of length to width of conidia arranged in classes.

have been followed. The writer makes no pretense to a comprehensive biometric study of the two Sclerosporas but has used this method solely as a means to the end of presenting the accompanying data as a basis of comparison between these and other species.

TABLE I.—*Summarized measurements of conidia of Sclerospora spontanea and Sclerospora philippinensis*

Length.			Diameter.			Length over diameter.		
Classes.	Number of conidia in 700.		Classes.	Number of conidia in 700.		Ratio classes.	Number of conidia in 700.	
	S. spontanea.	*S. philippinensis.*		*S. spontanea.*	*S. philippinensis.*		*S. spontanea.*	*S. philippinensis.*
μ.			μ.					
17 to 18.9		1	11 to 12.9	11	2	0.95 to 1.14		
19 to 20.9		1	13 to 14.9	175	18	1.15 to 1.34		2
21 to 22.9		2	15 to 16.9	391	119	1.35 to 1.54		5
23 to 24.9		5	17 to 18.9	121	321	1.55 to 1.74		41
25 to 26.9	1	17	19 to 20.9	2	199	1.75 to 1.94		154
27 to 28.9	3	27	21 to 22.9		50	1.95 to 2.14	7	221
29 to 30.9	10	77	23 to 24.9		1	2.15 to 2.34	28	163
31 to 32.9	12	134				2.35 to 2.54	92	88
33 to 34.9	20	153				2.55 to 2.74	102	24
35 to 36.9	93	107				2.75 to 2.94	154	8
37 to 38.9	76	75				2.95 to 3.14	161	4
39 to 40.9	87	39				3.15 to 3.34	88	
41 to 42.9	112	29				3.35 to 3.54	37	
43 to 44.9	94	23				3.55 to 3.74	22	
45 to 46.9	79	7				3.75 to 3.94	5	
47 to 48.9	46	2				3.95 to 4.14	3	
49 to 50.9	22	0				4.15 to 4.34	0	
51 to 52.9	15	1					1	
53 to 54.9	12							
55 to 56.9	7							
57 to 58.9	5							
59 to 60.9	4							
61 to 62.9	0							
63 to 64.9	2							

TABLE II.—*Biometric constants of the conidia of Sclerospora spontanea and Sclerospora philippinensis*

LENGTH

Species.	Mean.	Median.	Mode (approximate).	Standard deviation.	Coefficient of variability.
	μ.	μ.	μ.		
S. spontanea	42.07±0.145	41.86±0.142	41.43	5.672±0.102	13.48±0.247
S. philippinensis	34.52± .113	34.12± .142	33.32	4.439± .080	12.86± .335

DIAMETER

	μ.	μ.	μ.		
S. spontanea	15.79±0.036	15.84±0.045	15.93	1.395±0.025	8.83±0.160
S. philippinensis	18.40± .047	18.36± .006	18.36	1.834± .033	9.97± .181

RATIO OF LENGTH TO DIAMETER

S. spontanea	2.71± .009	2.71±0.011	2.71	0.357±0.006	13.20±0.242
S. philippinensis	1.91± .007	1.89± .008	1.85	.266± .005	13.92± .256

An examination of the data shows clearly that the long-spored Visayan form, *Sclerospora spontanea*, at least in regard to its conidia, is quite distinct from *S. philippinensis*. The location of the two frequency curves shows that the great bulk of the conidia of *S. philippinensis* fall between the limits of 31 to 36.9 μ in length, and 17 to 18.9 μ in width; while, on the contrary, a like proportion of those of *S. spontanea* are 37 to 46.9 μ in length and 15 to 16.9 μ in width. The somewhat irregular character

of the length curve of the latter species does not, in the opinion of the writer, indicate that it is bimodal, because, by using more inclusive measurement classes of 4 μ or even 3 μ, the depression so noticeable with the 2-μ classes smooths out and the curve becomes quite regular. Moreover, the difference between the modes as well as between the means and the medians is still sufficiently great to emphasize strikingly the dissimilarity in size of the conidia of the two species.

It should be noted that, although the curves of frequency distribution of the two species overlap slightly, size is none the less a valuable diagnostic criterion. In length, for instance, the curves overlap from 26 μ, the lowest limit of the Visayan Sclerospora, to 52 μ, the highest limit reached by *Sclerospora philippinensis*. As a result, it might be contended that size is of no value in distinguishing between the two species when applied at least to the conidia falling between these limits. While this is true of any one conidium, experience shows that, if several are measured, exceedingly few are to be found in this disputed region. For practical purposes even 50 unselected conidia of each species are sufficient to show the difference between them without any confusion due to overlapping.

It is also worthy of note that the curves of the frequency distribution of 700 conidia in both the Visayan species and *Sclerospora philippinensis* differ in no essential particular from those of 500, 400, or even as few as 200 conidia.

Furthermore, in the ratios of length to width of their conidia, the two species also show marked differences. The shorter, broader spores of *Sclerospora philippinensis* most commonly show ratios of 1.55 to 2.14, while in *S. spontanea* the greater length as well as the lesser width of the conidia is expressed by the predominant ratios of 2.35 to 2.94.

In order to determine whether the differences between the biometric characteristics of the two forms were indeed significant, the method quoted by Rosenbaum (*11*) from Reitz and Smith was employed. This method, which compares the difference between the mean or other constants with the probable error of the difference, shows that in *Sclerospora philippinensis* and *S. spontanea* these differences without doubt are significant and can not be the result of mere random sampling. This significance is clearly brought out in Table III.

TABLE III.—*Difference in means of Sclerospora spontanea and Sclerospora philippinensis compared to the probable errors*

Difference in means.			Difference in means divided by probable error of difference.		
Length.	Diameter.	Length over diameter.	Length.	Diameter.	Length over diameter.
μ 7.55±0.183	μ 2.61±0.058	0.798±0.011	41.27	44.96	70.39

The identity of the long-spored, Visayan Sclerospora, then, is clearly established as quite distinct from *Sclerospora philippinensis*. Whether this distinction is sufficient to entitle the former to specific rank depends somewhat upon the judgment of the investigator. The matter could be settled with greater finality if the two fungi were to be grown in pure culture and compared in morphological and physiological details under the controlled conditions of the laboratory, but unfortunately all attempts to grow the two forms artificially have been unsuccessful. In view, however, of such well-defined, although somewhat relative, morphological differences in the conidiophores as the peculiarities of the basal cell and the branch system, and the well-marked and easily measurable differences in size and shape between the conidia of the two fungi, and in view of the constancy and persistence of these points of dissimilarity over a wide range of hosts, through several generations of maize and during three months' cultivation, the writer regards the Visayan form as worthy of specific distinction from *S. philippinensis*. The species, therefore, is described as new, and as it was first found occurring spontaneously on a wild host, it is named *S. spontanea*.

DIAGNOSIS

Sclerospora spontanea, n. sp.

Symptoms, effect on the individual host, and destructiveness to the maize crop as a whole, as previously described by the writer for *Sclerospora philippinensis* (12).

Mycelial hyphae and haustoria as described for *Sclerospora philippinensis;* but the clavate hyphae (conidiophore initials) which emerge from the stomata are longer, more slender, and more irregular.

Conidiophores as in *Sclerospora philippinensis*, erect, single or grouped, developing only at night and in dew; comprising basal cell, main axis, more or less complex dichotomous branching system, and terminal sterigmata; but differing in general in greater total length (350 to 550 μ) and more expanded top, and in particular as follows: Basal cell less knobbed and expanded at the base, more slender (least diameter about 5 to 8 μ), and longer (140 to 260 μ), usually exceeding or at least equaling in length the extent of the main axis from the septum to the primary branches. Main axis usually expanding more abruptly above the septum to a greater width (22 to 32 μ) and constricting noticeably (to about 20 μ) below the branches. Branches longer, more slender, less constricted at point of origin, less recurved and ascending, but standing out more stiffly. Sterigmata longer (13 μ), more slender, and straighter.

Conidia resembling those of *Sclerospora philippinensis* in hyaline, finely granular content, thin wall, rounded apex lacking papilla, and rounded base with apiculus of attachment, and in invariable germination by tubes; but differing as follows: In shape, longer and more slender, usually very elongately ellipsoid or cylindrical; in size, showing greater length and less width, the majority being 39 to 45 μ long by 15 to 17 μ in diameter.

Oospores not yet encountered on maize, although an oogonial stage on *Saccharum spontaneum* may prove to be connected.

HABITAT.—Found in the Visayan group of the Philippine Islands principally on cultivated maize (*Zea mays* L.), rarely on the wild grass bugangĕ (*Saccharum spontaneum* L.), and once on cultivated sugar cane (*Saccharum officinarum* L.). Inoculated successfully upon the first two of these hosts and also upon teosinte (*Euchlaena luxurians* Schrad.), and the wild grass *Miscanthus japonicus* (Thunb.) Anders. Extremely destructive to maize, but much less so to the other hosts.

Material of the type will be found in the pathologic collections of the Bureau of Plant Industry, Washington, D. C., and in the herbarium of the Bureau of Science, Manila, P. I.

DISCUSSION

RELATIONSHIP

The two Sclerosporas, *Sclerospora spontanea* and *S. philippinensis*, are undoubtedly closely allied to each other. It is even possible that future investigation will bring to light forms intermediate between them. Such may be the downy mildew on maize seen by Prof. Reinking in the Cotabato Valley and by Gov. Coverston in Lanao Province, both of which places are in the southern Island of Mindanao. On the other hand, the Mindanao form may be as different from *S. spontanea* and *S. philippinensis* as these have proved to be from each other. The writer feels confident that on further search additional Sclerosporas will be encountered in the Philippines both on cultivated hosts and on wild grasses.

The relationship of the Philippine downy mildew Sclerospora to the similar forms described on maize and related crops from other oriental countries has been discussed in connection with *Sclerospora philippinensis* (*12*). Unfortunately the matter can not be settled finally with the data available. As the writer's discovery that suitable material can be secured only at night is very recent, previous publications present measurements and other data inadequate for comparison with living material. In so far as one can judge, however, *S. spontanea*, on account of its longer, more slender spores, is even more sharply distinguished than is *S. philippinensis* from the Javan species, *S. javanica* Palm (*10*), from the species of British India, *S. maydis* (Rac.) Butl. (*1*), and from the normal, short-spored type of the Formosan species, *S. sacchari* Miyake (*9*). It is of interest to note, however, that in the greater length of its conidia, the very character wherein it differs so distinctly from these other oriental species, *S. spontanea* tends to resemble the two abnormally long-spored forms recorded by Japanese investigators. In his account of *S. graminicola*, Ideta (*8, p. 143–145*), in addition to conidia of the size characteristic of the species, mentions a class of conidia having the—

shape of a long ellipse, 38.4 to 57.6 μ long by 19.2 to 24 μ wide.

Also, Miyake (*9*), in his account of *S. sacchari*, describes conidia not only of the usual shape and size, but also of an unusual type—

long ovate, 49 to 54 μ by 19 to 23 μ.

The descriptions and drawings of both these long types of conidia remind one of the spores of *S. spontanea*, even though the latter are characteristically more slender. It is very probable that the occurrence of these long conidial types in Japan and in Formosa indicates the

existence there of strains or species of Sclerospora as yet unrecognized; but what their relationship and significance may be, future investigation must determine.

The relationship of these two Philippine conidial forms to the oogonial stage characteristic of the genus is as yet unknown. Whether *Sclerospora philippinensis* or *Sclerospora spontanea* is connected with the oogonial stage which is so common on *Saccharum spontaneum* throughout the Philippine Islands is yet to be established. The writer has attempted to germinate the oogonia of the latter and to obtain inoculations with them, but so far he has been unsuccessful. Until the precise connection is definitely established, it is well to be cautious about assuming that the two types of spores are with certainty different phases of the same species. It may be worthy of note that the writer has found, in addition to the oogonia on *Saccharum spontaneum*, similar spores on *Miscanthus japonicus* and on cultivated sugar cane in the mountains of northern Luzon. On all these hosts the oogonia are apparently the same species; and their significance and importance will be discussed by the writer in a later paper.

NONSPECIALIZATION

As the problem now stands, the Philippine maize-mildew presents an interesting situation, since it involves two causal Sclerosporas quite distinct morphologically but practically indistinguishable physiologically both in their effect on, and in their virulence to, a range of hosts. The genus Sclerospora seems, then, to present a marked contrast to the strong specialization of the closely related genus Peronospora. In the latter, the work of Gäumann (5, 6, 7) has shown that the species are strongly specialized, being distinct on different hosts. This is true especially in the Rubiaceae (7), but also to a marked degree in the Cruciferae (5) and the Scrophulariaceae (6). The distinction holds both morphologically, in the size and character of the conidiophores and conidia, and also physiologically, in their inability to infect any host species but that from which the spores were derived. Gäumann, therefore, regards it as highly improbable that the same host species would be found to harbor more than one species of Peronospora. In Sclerospora, however, we have the two species, *Sclerospora spontanea* and *S. philippinensis*, morphologically distinct, yet both with equal ease inoculating the same series of hosts, including members not only of the Maydeae but also of the Andropogoneae.

SIGNIFICANCE OF OCCURRENCE

The finding of *Sclerospora spontanea* on a wild gramineous host is of interest. Hitherto in spite of the attention which the destructive oriental Sclerosporas have attracted, no conidial representative of the genus has ever been reported as occurring naturally upon a wild host. It is a question whether the occurrence of *Sclerospora spontanea* on wild

Saccharum in the Visayan Islands should be regarded as throwing light on the problem of the origin of the Philippine downy mildews of maize. In the opinion of the writer this and other facts indicate that the native grasses of the Philippines were the original hosts from which the downy mildews passed and are passing to such very susceptible introduced crops as maize. On the other hand, one should not overlook the possibility that the wild Saccharum clumps might have been infected with the downy mildew from badly diseased maize growing near. In this connection it should be noted that in two cases where *Sclerospora spontanea* was found on wild bugang grass (*Saccharum spontaneum*) the infected clumps were so far distant and so protected from any downy-mildewed maize that there was little possibility of their having been infected thus. In the other cases the infected bugang clumps were much older than the mildewed maize adjacent; and, because inoculation experiments have shown that bugang grass is susceptible only as comparatively young seedlings, there is little doubt that the infection in the grass clump had been carried over in the perennial rootstocks and had not been caught from maize.

Moreover, it is worthy of note, also, that the wild Saccharum is very resistant to the effect of the Sclerospora, while maize is exceedingly unresistant. In contrast to the susceptibility to severe injury already noted in maize, wild Saccharum, even though heavily infected, shows only slight striping of the leaves (Pl. 78, B, C), remains undeformed, and is not materially retarded in development. In spite of the downy mildew the plants continue to grow vegetatively, to produce flowers (Pl. 77, B), and to form, by tillering, dense clumps which by extensive rootstocks persist from season to season, still supporting the active and equally persistent parasite. Because, as a rule, it is the introduced host which is most injured by a disease and the original, native host which is relatively unaffected, the indications are that wild Saccharum and not maize is the original host of *Sclerospora spontanea*.

The finding of *Sclerospora spontanea* on sugar cane is a second point of interest. Because, in Formosa, the closely related species *S. sacchari* Miyake had proved indiscriminately destructive to both sugar cane and maize, the writer, while in the Philippines, made especial effort to discover instances of the transmission of downy mildew from one to the other of these hosts. The single case in Cebu, however, was the only one noted. In this instance the single clump of sugar cane infected with *S. spontanea* was situated at the extreme edge of the field, separated only by a narrow trail from a large planting of badly downy-mildewed maize. Although the whole sugar-cane field was carefully inspected, no other cases of Sclerospora were discovered. It is natural to infer that the sugar-cane plant was infected from the neighboring maize, especially since the two parasites proved to be the same. It is rather surprising, however, that this lone cane plant, of all the thousands examined in scores of different

fields adjacent to or even interplanted with infected maize, should be the only one to succumb.

The matter is still further complicated by the fact that in Formosa Miyake easily obtained the infection of sugar-cane plants grown from cuttings, while in the Philippines the writer was not able to inoculate cutting-grown plants of sugar cane, or even of *Saccharum spontaneum*, although seedlings of this grass were readily infected (Pl. 78, A). Moreover, in Formosa the effect of *Sclerospora sacchari* Miyake on sugar cane is far more destructive than was the effect of *Sclerospora spontanea* on this single cane plant. In the former the elongation and weakening of the shoots and the conspicuous yellowish striping of the leaves are a distinct contrast to the stunting of the shoots and faint, pale green markings of the leaves which characterized the Philippine specimens. Also, although the latter died shortly after being transplanted, this was apparently due to the severe treatment they had received rather than to the destructive character of the Sclerospora. It is possible that *Sclerospora spontanea*, in its essential individuality, is much less virulent to sugar cane than *Sclerospora sacchari*, or it may be that some limiting factor is operative in the Philippines. The work of Fawcett (*4*) indicates that temperature differences may exercise an important limiting effect within a smaller geographic range than from Cebu to Formosa. In any case, although the matter is in need of further study, it can safely be said that in so far as has been observed in the Philippines the production of sugar cane is unaffected by *Sclerospora spontanea* or other conidial Sclerosporas.

SUMMARY

The downy mildew of maize which is extremely destructive in the Philippine Islands has been found to be caused by the Peronosporaceous genus Sclerospora. At first only one species was thought to be involved, and this was described by the writer as *Sclerospora philippinensis*. More recently the problem presented by the Philippine maize-mildew has been still further complicated, since a second causal species of Sclerospora has been found to be concerned also. The foregoing paper describes this species as new (*S. spontanea*) and presents briefly its morphological and physiological characteristics and its importance and relationship.

Sclerospora spontanea, the more recently discovered form, occurs in the Islands of Cebu, Bohol, and Leyte, where it was found on the wild grass *Saccharum spontaneum* L., on sugar cane (*Saccharum officinarum* L.), and on maize (*Zea mays* L.). *Sclerospora philippinensis*, the species first recognized, occurs in the Island of Luzon, where it was found on maize, teosinte (*Euchlaena luxurians* Schrad.), and sorghum (*Andropogon sorghum* [L.] Brot.).

Morphologically, *Sclerospora spontanea* is characterized by the relatively much greater length and slenderness of its conidiophores in general and of its basal cells and conidia in particular. In these respects it differs markedly from *S. philippinensis*, which has shorter, stockier

conidiophores, shorter, thicker basal cells, and shorter, broader conidia. There are, moreover, some minor distinctions between the branch systems and between the sterigmata of the two species.

These differences remain constant for each species and are not influenced by growth on different hosts even through several generations. Both species have been artificially inoculated with equal ease from one to another of the following hosts: Maize, teosinte, *Miscanthus japonicus*, and *Saccharum spontaneum*. Attempts to inoculate sorghum artificially were unsuccessful with both species. Because no seedlings of sugar cane were available, no inoculation with either fungus was attempted. Inoculations on sprouted sugar-cane cuttings were uniformly unsuccessful.

Since the size and shape of the conidia are the most useful criteria of interspecies distinction, they are given in detail. Measurements of 700 conidia of each of the two species were combined into comparative tables and graphs of frequency distribution in an attempt to present the differences between them quantitatively as well as qualitatively.

Although morphologically the two species differ as has been described, yet physiologically, in general effect in the field, in effect on the individual plant, and in virulence to the same wide range of hosts no distinction between them is apparent.

The discovery that two forms are involved complicates the problem presented by the Philippine downy mildew of maize. Because two forms morphologically different but practically indistinguishable in physiologic effect are concerned in the same disease, there appears to be a decided lack of that specialization which characterizes certain other genera of the Peronosporaceae. It seems highly probable that still other forms will be found to be concerned in similar diseases in the Philippine Islands and throughout the Orient.

In addition to these two conidial species with a host range of maize, teosinte, sorghum, sugar cane, *Saccharum spontaneum*, and *Miscanthus japonicus*, the writer has encountered in the Philippines oogonial stages of Sclerospora on *Saccharum spontaneum*, *Saccharum officinarum*, and *M. japonicus*. The oogonia on these three hosts are practically indistinguishable. Whether these oogonial and conidial stages are quite unrelated or are indeed only phases in the development of the same organism remains to be determined.

Sclerospora spontanea, like *S. philippinensis*, is closely related to the other conspicuous conidial Sclerosporas of the Orient: *S. javanica* Palm, of Java; *S. maydis* (Rac.) But., of India; and *S. sacchari* T. Miyake, of Formosa. All these forms are characterized by the predominance of the conidial stage, the absence or great rarity of the oogonia, germination of the conidia by tubes, and the occurrence on maize, sugar cane, and related hosts in the Orient. *S. spontanea*, however, because of its longer, more slender spores is as a species distinguished even more sharply than *S. philippinensis* from these other oriental representatives.

The discovery of *Sclerospora spontanea* on wild *Saccharum spontaneum* is, in so far as the writer is aware, the first record of the occurrence of a conidial Sclerospora on a wild host in the Orient. This occurrence, in connection with other data, seems to the writer to indicate that the wild grasses are the natural hosts of these oriental downy mildews from which they have passed and are passing to susceptible introduced crops such as maize.

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PLATE 76[1]

Corner of a native-grown maize plot in the interior uplands of Cebu. At the edge of this field, in which many maize plants were being killed by downy mildew, were occasional clumps of the wild grass (*Saccharum spontaneum* L.) called "bugang" in the Visayan Islands. One of these clumps, which was severely infected with *Sclerospora spontanea*, is shown at the left. The older, primary stalk of this clump, had died, but although the remaining shoots were apparently uninjured, great numbers of conidiophores were being produced on them, especially on the one held out for inspection. The base of this shoot was a few feet farther down the steep slope at the point indicated by the arrow. Behind the central figure can be seen a maize plant noticeably discolored by the downy mildew.

[1] Photographs by W. H. Weston.


A
B

PLATE 77

A.—Clump of *Saccharum spontaneum*, showing characteristic size and habit of healthy plants under natural conditions. The measure is 2 meters tall.

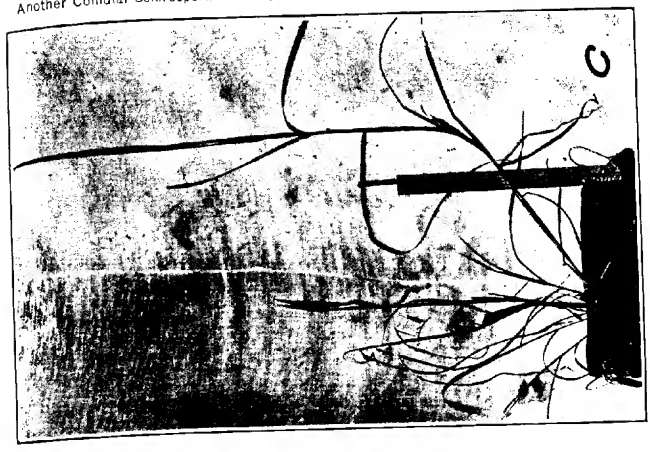
B.—Clump of *Saccharum spontaneum* infected with *Sclerospora spontanea*. When transplanted to this container in Cebu the infected plant comprised a single shoot separated from the clump shown in the preceding plate. This shoot continued to develop vigorously in spite of the downy mildew until after 5¼ months it had produced the thriving clump shown. Conidiophores were still being produced in abundance, especially by the younger stalks. Same measure as in A.

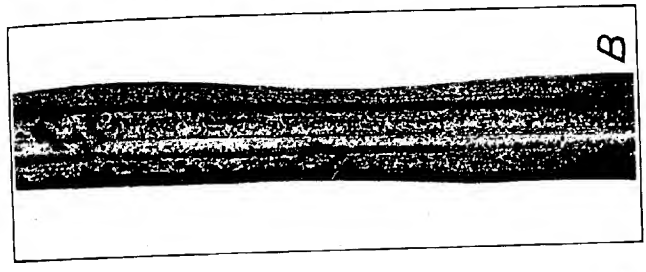
PLATE 78

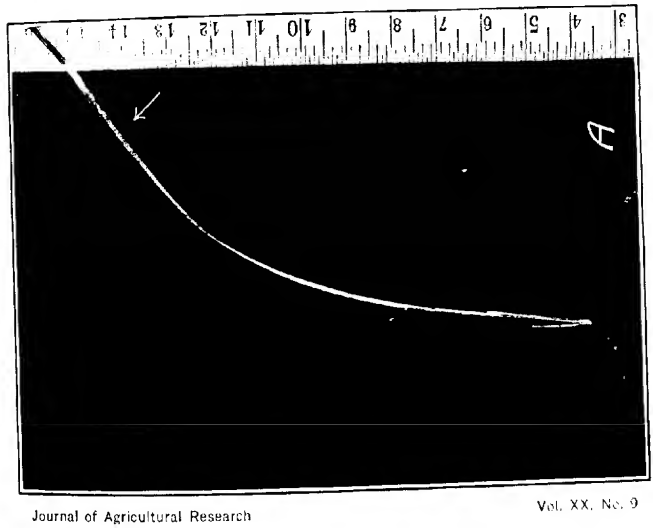
A.—A young seedling (3 weeks old) of *Saccharum spontaneum* infected with *Sclerospora spontanea*. On this seedling, which was artificially inoculated on the second night after it emerged, conidium production began on the sixth night following and recurred in increasing abundance on successive nights. In contrast to healthy seedlings this plant betrays the effect of the Sclerospora in its pallor and in the presence of a whitish "down" of conidiophores. These have collapsed on drying but can still be seen on that part of the fourth leaf indicated by the pointer. × ¾.

B.—Conidiophores on the leaf of *Saccharum spontaneum*. A portion of the upper leaf surface of a downy-mildewed plant (Pl. 77, B) showing remains of the whitish "down" of innumerable conidiophores produced during the night. Although photographed as early as light would permit, the leaf surface has dried somewhat and the fragile conidiophores have shrunk and matted together. × 1½.

C.—Young shoots of *Saccharum spontaneum* arising after the primary stalk had been cut, and like it severely infected with *Sclerospora spontanea*. The main plant, one of the four downy-mildewed ones transplanted from Cebu, was cut off close to the ground. All the subsequent shoots arising from the remaining base were, from the first leaf, badly infected with Sclerospora and produced abundant conidiophores.


C


B


A


A
B
C
D
E
F
G
H
I
J
K
Weston

PLATE 79 [1]

A.—Typical conidiophore,[2] showing characteristically long, slender, unknobbed basal cell, relatively short main axis with its greatest diameter about midway to the primary branches, and fairly well-developed branch system bearing long, slender conidia. The number of conidia is somewhat less than that usually encountered. From maize inoculated from *Saccharum spontaneum*. × 375.

B.—Upper portion of a conidiophore which has a poorly developed branch system and hence bears few conidia on sterigmata which are relatively large. Several conidia have been broken off in mounting. From maize. × 375.

C.—Portion of the branch system of a conidiophore, showing the conidia germinating while still attached to their sterigmata. From maize. × 375.

D.—Stalk portion of a typical conidiophore, showing long, slender, unknobbed basal cell, and main axis which is slender above the septum, expands rapidly to its greatest diameter about midway, and contracts again below the branches. From *Saccharum spontaneum*. × 375.

E, F.—Typical basal cells of conidiophores. E from *Saccharum spontaneum;* F from sugar cane. × 375.

G.—Stalk portion of a conidiophore with basal cell which, though unusually short, nevertheless is longer than the extent of the main axis from septum to primary branches. From *Saccharum spontaneum*. × 375.

H.—Typical stalk portion of a conidiophore from sugar cane. Compare with A and D. × 375.

I, J, K.—Typical conidia showing variations in size and shape and method of germination by hyphae. I from maize, the lowest figure from material especially fixed and stained to bring out the internal structure; J from *Saccharum spontaneum;* K from sugar cane. × 375.

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[1] The drawings were made with the aid of a camera lucida. Figure A and the ungerminated conidia of figures I, J, and K are from fresh material. All the other drawings are from preserved specimens.

[2] In comparing these drawings with the plates of *Sclerospora philippinensis* (12) it should be noted that the latter give a somewhat misleading impression of the relative spreading of the branch system because the conidiophores were flattened slightly in mounting.

ONION SMUDGE

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INTRODUCTION

Smudge is a common disease of onions occurring both in the field and in storage or transit. It is confined for the most part to the bulbs and is characterized by dark green to black spots of variable size and shape on the outer scales. The spots may be homogeneous in appearance or may consist of numerous individual stromata scattered miscellaneously or arranged in concentric rings. The disease is most common on the white varieties of onions and damages materially the appearance and market value of the crop. The causal fungus has heretofore generally been known as *Vermicularia circinans* Berkeley, but as explained later in this paper it should more properly be termed *Colletotrichum circinans* (Berk.) Voglino.

The present investigations have been carried on with special reference to the disease as it occurs in the districts of southeastern Wisconsin and northeastern Illinois where onion sets are grown. The growing of white onion "bottom sets" is an industry of considerable importance in these sections, and the methods used in growing and handling the set crop are often conducive to the excessive development of smudge during and immediately following harvest. In this study attention has been given primarily to the mycological and physiological aspects of the causal organism, the relation of the parasite to the host tissue, the life history of the fungus with relation to the production of disease, and the development of remedial measures.

THE DISEASE

COMMON NAMES

A number of common names have been used in American and European literature for this disease—namely, "onion Vermicularia" (*3*)[2], "Vermiculariose" (*29*), "black spot" (*7*, *30*), "scab" (*17*, *21*), "anthracnose" (*7*, *36*, *37*, *38*), and "smudge" (*26*). The name "anthracnose"

[1] This study was begun in the Department of Plant Pathology at the University of Wisconsin in 1914, and the major portion was completed in 1917. Since the writer entered the Office of Cotton, Truck, and Forage Crop Disease Investigations in the latter year, observations have been extended to sections outside of Wisconsin. Grateful acknowledgments are expressed to Dr. L. R. Jones, under whose immediate direction the work has been done, and to Drs. J. J. Davis and E. M. Gilbert, who have given valuable aid and suggestions on the mycological phases of the problem.

[2] Reference is made by number (italic) to "Literature cited," p. 719–721.

has been much used up to the present time. However, since the symptoms have little in common with those of the more common anthracnoses, and since it is believed that as simple and as descriptive a name as possible should be chosen, the name "onion smudge" is used in this paper to designate the disease, and this name is recommended for general usage.

HOST PLANTS

White varieties of the onion (*Allium cepa*) are the chief ones affected by smudge, but all varieties thoroughly tested have been found susceptible to at least a slight degree. The disease also occurs on shallots (*A. ascalonicum*) and on leek (*A. porrum*). It has never been found on garlic (*A. sativum*).

HISTORY AND GEOGRAPHICAL DISTRIBUTION

Onion smudge was first described in 1851 by Berkeley (*4*) in England, where it was found on the outer scales of a white variety. Subsequent reports of its occurrence in Europe have been made by Massee (*17*) in England, Bubák (*8*) in Bohemia, and Voglino (*35*) and Allescher (*1*) in Italy.

The first collection of this disease in America, made by Michener, was reported by Berkeley (*5*) in 1874. Since that time it has been recorded in literature as occurring in Rhode Island (*3*), Connecticut (*10, 19, 33*), New York (*20, 22*), New Jersey (*13, 25*), Ohio (*26*), Indiana (*21, 34*), Illinois (*30*), Wisconsin (*23*), and Alabama (*2*). Additional data furnished by the Plant Disease Survey show that it has been present also in Massachusetts, Pennsylvania, Delaware, Maryland, Virginia, Georgia, Louisiana, Texas, Minnesota, and Iowa.

It is thus a disease of widespread occurrence; and, indeed, when one considers the fact that thousands of bushels of infected "bottom" sets are being shipped annually to all parts of the country and abroad, it is reasonable to suppose that its distribution is even more general than this summary indicates.

DESCRIPTION OF SMUDGE (PL. 80, 81)

The disease is confined entirely to the scales and the lower portions of the unthickened leaves which constitute the neck of the bulb. It first becomes manifest upon the appearance of minute stromata which form just beneath the cuticle of the host. These are dark green at first, becoming black with age. Depending on conditions of infection, the individual stromata may be scattered miscellaneously over the surface of the bulb, or, as is more commonly the case, they may be congregated in smudgy spots around a few centers of infection. These spots are usually roughly circular and variable in size. They often coalesce and occasionally contain stromata arranged in concentric rings. Under moist conditions the stromata bear acervuli which contain prominent setae readily distinguished with a lens of low magnification. Cream-colored spore masses frequently form on these fruiting bodies.

Penetration of underlying dry scales by the fungus causes similar spots, which are commonly surrounded by yellowish borders. On the fleshy scales the disease first appears as minute, sunken, yellowish spots which gradually enlarge and often coalesce. As the disease progresses, the black stroma of the fungus usually appears; and, with the collapse of the host cells, spots very similar to those on the dry outer scales result. When the dark-colored stroma does not develop before the scale has entirely dried down, the affected portions appear as slightly raised, yellowish spots, giving to white onion sets an unnatural color which is almost as detrimental to their market value as the black, smudgy spots.

The disease makes its appearance early in July under Wisconsin conditions, the fungus living on the outer dead scales and increasing in amount up to harvest time, when the outer two or three scales may be affected. From this time on it penetrates farther into the bulbs, progress depending upon environmental conditions. Badly diseased bulbs tend to sprout prematurely in storage. In most severe cases the fungus penetrates the entire bulb and causes a complete collapse of the fleshy scales.

The foregoing description applies to the disease as it appears on white onions. On colored varieties (red, yellow, and brown) the fungus is confined, with rare exceptions, to the neck of the bulbs where there is little or no pigment in the tissue, and the symptoms in these cases resemble closely those on the corresponding parts of the white varieties.

On shallots the disease appears as smudgy spots very similar to those on onion and is confined to the outer leaves or scales. On leeks similar symptoms prevail.

OTHER DISEASES LIKELY TO BE CONFUSED WITH SMUDGE

Onion bulbs as they mature are subject to attack by a number of fungi which develop saprophytically on the dead outer scales and produce symptoms which may easily be confused with those of smudge. The most common of these are two species of Macrosporium (*Macrosporium porri* Ell. and *M. parasiticum* Thüm.) (*33*), and a species of Phoma, probably *Phoma alliicola* Sacc. and Roum. (*24*). The Macrosporiums produce irregular, dark green spots which are due to ramification of the mycelium through the dead scales, but which lack the stromata and more or less regular outline of the smudge spot. In a moist atmosphere the fungi fruit and develop a dark green mold due to the production of conidia (Pl. 81, F, G). In rare instances black perithecia of *M. parasiticum* are found on the outer bulb scales. Phoma produces small black pycnidia which are often difficult to distinguish macroscopically from the stromata of the smudge fungus. It is commonly associated with *M. porri* (Pl. 81, H). These two fungi commonly attack both white and colored varieties, and in the latter case the pigment in the outer scales is usually destroyed, giving a symptom which is known in the trade as "onion blotch."

Onion smut is sometimes confused with smudge, especially when the former occurs on mature bulbs. In such instances, however, smut usually causes slightly raised, linear lesions which on colored varieties are commonly accompanied by more or less destruction of pigment. The exposure of the powdery spore mass upon breaking of the lesion establishes the identity of the smut fungus.

ECONOMIC IMPORTANCE

The importance of smudge as a detriment to the onion crop may properly be considered from three standpoints—(1) that of reduction of market value as a result of marred appearance, (2) that of actual shrinkage of the bulbs in storage, due to fungus invasion, and (3) that of increased sprouting of onion sets during storage. Thaxter (*33*) calls attention to the reduction of market value caused by smudge, citing an estimate by one grower of an actual loss of several thousand dollars to his crop in one season on this account. There is little doubt that marked spotting by this disease hampers greatly the disposal of white onions, since they are usually grown at a greater expense than colored varieties for a fancy trade which is prone to discriminate against disfigured stock. Under prolonged storage smudge causes a distinct shrinkage of the bulbs and promotes premature sprouting. These last two factors are not usually of material importance on large bulbs, but they are of much significance with respect to onion sets. The latter are usually harvested in August and September and kept in storage until March. The small bulbs are thus subjected to fungus invasion for several months, and data presented later in this paper show that in badly diseased sets the shrinkage may be doubled by smudge during this period.

Sets which sprout badly during storage are a total loss to the owner, since they will not stand shipping and must be discarded. Much of the sprouting of white sets in storage is due to severe attacks by smudge. Experimental data in support of this statement are given later in this paper.

It will be seen, therefore, that smudge is of greater importance than would be suspected from casual observation. In the Chicago district alone, where approximately 1,000,000 bushels of sets are grown annually, the aggregate loss due to shrinkage in weight and sprouting probably runs into many thousands of dollars.

CAUSAL ORGANISM

MORPHOLOGY

The morphology of the causal organism has previously been discussed by Berkeley (*4*), Thaxter (*33*), Stoneman (*32*), Stevens and True (*30*), and Kempton (*16*).

MYCELIUM.—The mycelium ranges from 2 to 8 microns in width, is septate and branching, varying widely with age as to color and size. It

is at first hyaline with few septa, but later the walls thicken and take on a dark green color, oil droplets become more numerous, and septation is more frequent.

STROMATA.—By close intertwining of the thick-walled mycelial threads, dark green to black stromata, usually only a fraction of a millimeter in diameter and few to several hundred microns thick, are formed beneath the cuticle of the host (fig. 1). On nutrient media these stromata commonly coalesce, forming a black stromateoid layer at the surface of the substrate. This coalescence sometimes occurs on the host, but more often the stromata remain distinct and are connected with one another by threads of the dark-colored mycelium. During protracted storage, or under poorly ventilated conditions, excessive stromatal development may occur (Plate 83, B). Thaxter (*33*) describes large, somewhat flattened sclerotia, "jet black externally and white within,"

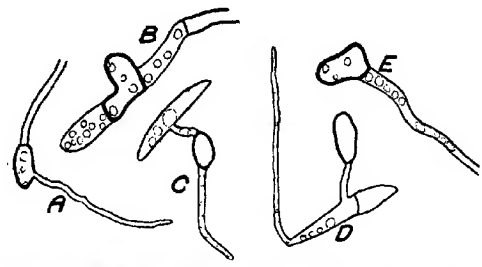

FIG. 1.—Conidia and appressoria of *Colletotrichum circinans*. The fusoid conidia (*C*, *D*) germinate by one or more germ tubes, often becoming septate during the process (*D*). Dark-colored, thick-walled appressoria develop at the tip of the germ tubes, usually as the latter come in contact with the host cuticle (*C*, *D*). Subsequent germination of appressoria commonly occurs (*A*, *C*). Terminal or intercalary appressoria-like cells, or chlamydospores, commonly develop within infected scales (*B*, *E*). Camera-lucida sketch. × 750.

associated with the disease, though he does not definitely state that they are connected with the causal organism. The writer has never found bodies of this sort connected with the disease. On the other hand, sclerotia of *Botrytis* spp., which cause decay of onion bulbs and are commonly associated with smudge, compare favorably with his description.

APPRESSORIA OR CHLAMYDOSPORES.—(Fig. 1). These bodies are variable in size, dark brown in color, thick-walled, egg-shaped or roughly circular, usually terminal but occasionally intercalary. In germination drops on glass slides they form most abundantly where the germ tube comes in contact with the slide and less commonly in the upper region of the drop. Under such conditions they measure 6.5 to 8 microns by 4 to 5.5 microns. In Petri-dish cultures on various types of nutrient agar they are almost invariably produced at the tips of hyphae which come into contact with the glass surface. When "infection drops" containing

viable conidia are placed on the surface of onion bulbs, appressoria or chlamydospores are formed in contact with the scale. Later they send out germ tubes which penetrate the host. They are also commonly found within the tissue of affected scales.

ACERVULI.—The fruiting bodies are formed on the stromata which develop beneath the cuticle of the host. Short, hyaline conidiophores form in a palisade layer and rupture the cuticle of the host (fig. 2). One to several acervuli form on a single stroma. In the study of the morphology of the fruiting body the writer has found no evidence of a closed or partially closed receptacle, as described originally by Berkeley (*4*). Its true nature is more nearly in accord with the work of Stoneman (*32*), who found not a pycnidium but an open fruiting body.

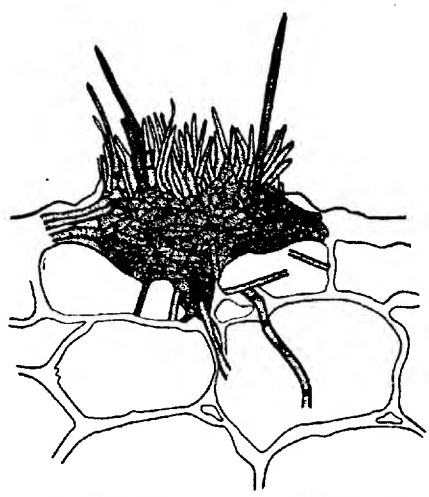

FIG. 2.—Acervulus of *Colletotrichum circinans* on artificially inoculated onion scale. Note the development of the stroma in the subcuticular wall and the rupture of the cuticle by the formation of the palisade layer of the sporiferous hyphae. Camera-lucida outline. × 265.

SETAE.—Scattered throughout the acervulus are numerous setae arising from the basal stroma. They are thick-walled, dark-colored, 0 to 3 septate, upwardly attenuate, and 80 to 315 microns in length.

CONIDIA.—The conidia are borne acrogenously, being budded off one at a time. They are fusiform, continuous, hyaline to slightly ochraceous, somewhat curved, and obtuse at the very apex. Typically one prominent vacuole is present in the center of the conidium, but under some conditions the cytoplasm may contain many large vacuoles. As the spores are budded off from the conidiophores they form a cream-colored, somewhat mucilaginous mass on the top of the fruiting body. The spores vary from 14 to 30 microns in length and from 3 to 6 microns in width. A large majority, however, fall within the limits of 18 to 28 microns by 3 to 4 microns. They germinate usually by one, but occasionally by two or

three germ tubes, which are pushed out at any point on the surface. Septation of the spore commonly occurs during germination.

PERITHECIA.—Stevens and True (*30*) report the development of an ascigerous form on onion sets heavily infected with *Colletotrichum circinans* and have referred the same to the new genus Cleistothecopsis. The writer has never been able to prove *C. circinans* to be connected with any ascigerous form found on onion. Stevens and True claim the connection between the perithecia of Cleistothecopsis and *C. (Volutella) circinans* on the following evidence:

(1) they occurred on sets badly infected with the Volutella; (2) no other fungi or other types of mycelium were seen to be connected with them; (3) when studied in various stages of development, the typical Volutella mycelium, which offers definite characters for recognition, was seen in organic connection with them, as illustrated in figure 18 (1), (4) the outgrowths from the perithecia are like those of the Volutella.

This evidence is hardly sufficient to prove that the two forms are stages of the same fungus, especially since a large number of saprophytic or semi-saprophytic forms very commonly occur on the dead outer scales of onion bulbs and the differentiation of these from C. *circinans* on the basis of the characters of the mycelium is sometimes very difficult. The writer has, therefore, considered it advisable to use the binomial of the imperfect form until cultures from a single ascus or ascospore of the ascigerous form are shown to be identical with *C. circinans* both as to morphological characters and pathogenicity upon onion bulbs.

TAXONOMY

The taxonomic questions involved in this study concern first, the proper position of the fungus in the present system of classification, and second, the possible identity of the organism with other described species.

Berkeley (*4*) in the original description of the fungus refers to the fruiting body as a perithecium and places it in the genus Vermicularia, giving it the name *Vermicularia circinans*. Thaxter's (*33*) description implies that the fungus has an open fruiting body, but he states that in the early stages of its development a "sort of membrane" extends over the basidia. Miss Stoneman (*32*) describes a thick basal stroma bearing an open fruiting body. She also suggests that the characters of the fungus resemble more closely those of the genera Colletotrichum and Volutella than of Vermicularia. Voglino (*35*), believing the fruiting body to be an acervulus, which would thus place the organism in the order Melanconiales, transferred the species to the genus Colletotrichum. However, he gives no report of any study of the formation of the fruiting body.

Stevens and True (*30*) in discussing the fungus describe a sporodochium consisting—

of a pseudoparenchymatous inner tissue covered by a continuous surface layer...The young sporodochium eventually ruptures its covering membrane...In all cases the conidiophores are borne upon a raised superficial base which constitutes the sporodo-

chium, in contradistinction to the innate form of the acervulus which has no such base. The tubercular swelling, due to the massing of mycelium below and in the epidermis, partakes of sporodochial character also, and while this subepidermal part may not be regarded as constituting a true sporodochium it serves to emphasize the tendency of the fungus to produce such structures... The structure is a tubercle with a differentiated cortical outer layer. This outer layer ruptures and the tubercle develops as a sporodochium... These facts exlcude the fungus from Vermicularia and place it in the Tuberculariaceae under Volutella.

In the discussion later in this paper on the relation of the parasite to the host it is shown that the development of the fungus commonly begins in the outer wall of the epidermal layer of host cells. As the cellulose becomes softened the hyphae multiply and a definite stroma forms within this softened cell wall. Mycelium penetrates the epidermal and underlying cells, and if humid conditions prevail the stroma will soon occupy several layers of subepidermal cells. In good storage this process is comparatively slow, but during a protracted period, especially if the humidity rises considerably from time to time, the stroma commonly does acquire a thickness of several hundred microns. An examination of many sections has shown that regardless of the extent of its development the stroma is always covered by the cuticle of the host. At the instant of sporulation a palisade layer of hyaline hyphae interspersed with dark-colored setae arises from the stroma, and in this process the cuticle is ruptured. This is shown to occur on stromata of widely different ages in figure 1 and Plate 83, B. It is to be noted in the first illustration that the stroma is of recent development, that it is confined to the outer wall of the epidermal layer, and that the cuticle has been ruptured only by the formation of the acervulus. In the second illustration, although the stroma is much greater in extent, the host cuticle is still to be found intact except where it has been ruptured by the two acervuli.

As pointed out by Saccardo (*24, v. 3, p. 221–222, 233*), certain species of Vermicularia are characterized by imperfect or cup-shaped pycnidia, and such forms approach the genus Colletotrichum. Obviously it is often difficult to determine the exact nature of the fruiting bodies, and as a result many forms belonging in Colletotrichum have been placed in Vermicularia. In the form under consideration there is no suggestion of pycnidial development at any time during the development of the fruiting body. On the other hand, it does fall within the limits of the genus Colletotrichum. It is true that the basal stroma is much more highly developed than in many of the better-known species of this genus. However, well-developed stromata have been described in several species of this genus, including *Colletotrichum antirrhini* by Stewart (*31*) and *C. cereale* by Selby and Manns (*27*). In both cases the stroma develops beneath the cuticle, which is ruptured only upon the formation of the acervulus.

It is quite possible that a critical study of the closely related species classified at present in Vermicularia and Colletotrichum will lead to the separation into another genus of those forms which develop acervuli above

thick basal stromata. This question, however, is not within the province of the present paper. Those species of the Hyphales which are placed in the family Tuberculariaceae are characterized by the grouping together of the sporiferous hyphae in a superficial, conglutinate, sessile, or stipitate mass, known as a sporodochium (*24, v. 4, p. 635, 682*). As already pointed out, Stevens and True (*30*) considered the fruiting body of the onion smudge organism to be of this nature and on that basis have transferred it to Volutella. In their description and figures, however, they seem to have interpreted the host cuticle as part of the so-called tubercle and thus as being of fungus origin. Were this true, the stroma would be superficial, and the fungus would properly belong to the genus Volutella. However, since the stroma is always subcuticular and the sporiferous hyphae are subcuticular in origin, the form is more characteristic of Colletotrichum than of Volutella. Here again it is obvious that these two genera need more critical study before their limits can be satisfactorily defined. Meanwhile in the light of evidence just given, the writer considers it more suitable to use the name *Colletotrichum circinans* (Berk.) Voglino for the onion smudge organism.

The comparison of *Colletotrichum circinans* with other related species has been very limited in this investigation. The list of species of this genus which coincide closely with the one in question as to spore measurements and general characters is large and extends over a wide host range. Obviously the comparison of herbarium specimens is insufficient basis for final conclusions under the circumstances. Critical comparison has been confined to *C. fructus* (S. and H.) Sacc., described as causing a fruit rot of apple. This species was originally described as a species of Volutella (*28*), but it was later transferred to Colletotrichum by Saccardo (*24, v. 13, p. 1201*)—

on account of the black setae and the acervulus being originally subcuticular.

Cross sections of apple fruits affected with *C. fructus* and with *C. circinans* are compared in Plate 83, C, D. In both cases the development of the stroma beneath the cuticle, which is ruptured only upon the formation of the acervuli, is clearly shown. The former species was chosen for comparative study because the spore measurements and general characters as previously described were closely similar to those of the onion smudge organism and authentic cultures were available.

Cultures of the apple organism or diseased fruits were secured from Prof. C. R. Orton, State College, Pa., Dr. L. R. Hesler, Ithaca, N. Y., Dr. Charles Brooks, Washington, D. C., and Mr. G. A. Meckstroth, Columbus, Ohio. Cross inoculation on apple and onion showed that *Colletotrichum circinans* was able to produce a rot of apple fruit similar to that produced by *C. fructus* (see Pl. 84, C). The formation of stromata and acervuli by both species on apple is shown in Plate 83, C, D. The rate at which the rot progressed, however, was uniformly slower in *C. circinans*. On onion,

C. fructus developed on the dead outer scale of the bulb, but no evidence of further invasion as occurs with *C. circinans* was observed. Thus, the two species are distinct as to pathogenicity.

Measurement of many hundreds of spores of several strains of both species produced on several substrates including the natural ones—namely, apple and onion—showed that the variations due to differences between strains and substrates along with differences due possibly to slight changes in environmental conditions precluded any distinction on this basis. The slight difference in the shape of spores shown in figure 3 was quite uniform. The spores of *Colletotrichum fructus* have walls nearly parallel throughout the middle half, and one end narrows much more abruptly than the other.

A comparison of growth on potato agar gave further evidence as to the distinction of the two species. The chief points of difference in development on this medium are as follows: (1) *Colletotrichum fructus*

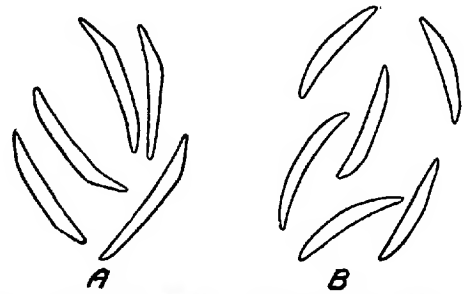

FIG. 3.—Spores of *Colletotrichum fructus* (*A*) and *C. circinans* (*B*). Note the slight difference in shape. In longitudinal section the walls of *C. fructus* are the more nearly parallel throughout the middle half, while at one end they converge more abruptly. Camera-lucida sketch. × 750.

grows the more rapidly, (2) appressoria at the tips of hyphae coming in contact with the glass surface in plate cultures are absent in *C. fructus*, (3) the method of branching is quite distinct—that of *C. circinans* is dichotomous while that of *C. fructus* tends to be monopodial in that nearly straight threads of mycelium, which become dark-colored very early and are greater in diameter, run out radially from the center of the colony and send out hyaline side branches of less diameter. Stromata develop at various points from these radial hyphae. This mode of growth gives a somewhat stellate macroscopic appearance to the colony, which differs from that of *C. circinans*, where distinctly radial hyphae are absent and stromata are scattered. This macroscopic difference is shown in Plate 84.

Thus, although the morphological characters are only slightly variant, the two forms are considered distinct (1) because of difference in pathogenicity, (2) because of difference in spore shape, and (3) because of difference in type of colony on potato agar.

PHYSIOLOGY

ISOLATION OF THE FUNGUS

Pure cultures of the causal organism are readily obtained by the ordinary spore-dilution method. On potato-dextrose agar colonies appear in three to five days. Single spore strains were isolated from such cultures by means of the method described by Keitt (*15*). Isolations thus made from many lots of diseased material collected in Wisconsin, Illinois, Ohio, Connecticut, and Louisiana have yielded strains which are closely similar in their behavior.

CULTURAL CHARACTERS

ON POTATO AGAR (2 PER CENT DEXTROSE) PLATES.—(See Pl. 84,D, E.) The conidium germinates within 6 to 8 hours, sending out one to three hyaline germ tubes, which within 24 hours are many times the length of the spore. Colonies become macroscopic in about 2 days. The mycelium becomes somewhat thicker and denser in the center of the colony, while the younger hyphae around the outer edge are thin-walled and hyaline. Those branches of mycelium which come in contact with glass plates usually produce dark-colored, thick-walled chlamydospores or appressoria. Within 2 or 3 days stromata begin to form by abundant branching from a definite point in the mycelium, which finally results in a thick mass of hyphae. These hyphae assume an olivaceous color, and by the fourth day the dark green stromata are macroscopic in size. They form first at the center and later throughout the colony except at the extreme outer edge. Occasionally they are arranged in such a manner as to give the appearance of "fairy rings," but this is not a constant characteristic. The appressoria and the stromata give the young colony an olivaceous appearance. It becomes darker and almost black with age as the stromata become denser and more numerous and finally form an almost homogeneous stromateoid layer at the surface of the substrate.

By the second day the colony shows a small amount of white aerial mycelium. This increases somewhat with age and later takes on a smoky gray appearance, masking the stromateoid layer to a certain extent. In from three to five days fruiting bodies are formed on the stromata at the center of the colony, and they continue to develop as the colony grows. Conidia are produced in abundance in most strains, accumulating in cream-colored or pinkish masses on the fruiting bodies.

The colony will continue to grow to an indefinite size if space and nutrients are available. A diameter of about 25 mm. is reached in seven days at room temperatures.

ON POTATO AGAR (2 PER CENT DEXTROSE) SLANTS.—Growth is similar in most respects to that on plates. Aerial mycelium tends to be more abundant. Mycelium does not, as a rule, extend deeply into the agar to form stromata. As the culture dries out the aerial mycelium forms a

dense mat over the surface of the culture, its color usually becoming slightly brownish with age. Spore masses often appear above this layer of mycelium.

ON OTHER MEDIA.—The growth of the fungus was studied on 25 kinds of artificial media, including beef broth agar, corn meal agar, oat agar, apple agar, synthetic agars, vegetable agars, cooked vegetables, and fresh vegetable tissues. The character of growth on the various media used was so uniform and so closely parallel to that on potato agar that a separate description for each is unnecessary. The most noticeable difference was that correlated with the supply of sugar in the medium. Where dextrose was omitted in the formula growth and sporulation were very scanty, and the stromata were few in number and widely scattered. On onion and apple agars made up without dextrose this difference was less marked, probably on account of the presence of a considerable amount of sugar in the plant tissues used. On synthetic agars [1] with sugar added in the form of maltose, dextrose, lactose, and sucrose copious growth took place with no evidence of preference for any one of the carbohydrates used. Cooked bean pod, onion scale, carrot, potato, and rice supported good development of the organism. On fresh onion and apple, however, the growth was much retarded, and on fresh potato and carrot it was very scanty. Stevens and True (*30*) report retarded growth on onion broth agar made with red or yellow varieties. The writer has found equally vigorous development on agar made from red, yellow, and white types of onion.

RELATION OF TEMPERATURE TO GROWTH

Potato agar plates inoculated with mycelium or conidia of the fungus were kept at temperatures ranging from 1° to 35° C. The rate of growth was determined by measuring the diameter of the resulting colonies or thalli from day to day. In order to increase the accuracy of the results Petri dishes of equal diameter containing equal amounts of agar were used. In order to overcome the influence of variations in relative humidity prevailing in different incubators the later experiments were modified by placing the Petri dishes in moist chambers first and then exposing them to the desired temperature. It was found after many trials that the best comparative data could be secured at four to six days. The growth was slight at 1°, almost negligible at 2°, but an appreciable amount occurred at 8° to 10° during a period of 10 to 14 days. Above this point the rate of growth increased rapidly, reaching the optimum at about 26°. At 31° to 32° little or no growth occurred on potato agar. The growth at various temperatures on this medium at the end of 6 days is represented graphically in figure 4.

[1] Formula for synthetic agar used: Sugar, 100 gm.; peptone, 20 gm.; ammonium nitrate, 10 gm.; magnesium sulphate, 2.5 gm.; potassium nitrate, 5 gm.; acid potassium phosphate, 2.5 gm.; calcium chlorid, 0.1 gm.; agar, 20 gm.; neutralized with normal sodium hydroxid.

A similar study of growth in tubes of onion decoction was made, with essentially parallel results. The optimum on this medium appeared to be slightly higher (27° to 29° C.) and slight growth occurred at 31°.

SPORE GERMINATION

RELATION OF MEDIUM.—For the studies upon spore germination a few drops of the liquid medium to be used were placed in Van Tieghem cells. A suspension of conidia in the same liquid was made, and a drop of this was transferred to cover glasses, which were then inverted over the cells and partially sealed with vaseline. The preparations were placed in Petri dishes and exposed to the desired conditions. For some purposes open drops on glass slides placed in Petri dishes lined with moistened filter paper were more suitable.

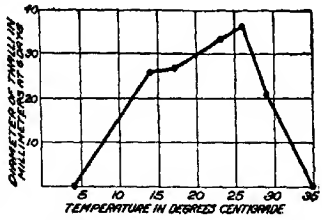

FIG. 4.—Relation of temperature to growth of *Colletotrichum circinans* on agar plates.

A comparative study of spore germination in distilled water, onion decoction,[1] onion leaf extract,[2] onion scale extract,[3] soil extract (sterilized and unsterilized),[4] and soil decoction [5] was made.

At room temperature germination in favorable liquid medium began within 5 to 6 hours. At 24 hours practically all viable spores had germinated. The percentage of germination in the drops was determined by averaging the counts of several microscopic fields. The results of these tests are summarized in Table I.

TABLE I.—*Effect of various media upon spore germination of Colletotrichum circinans*

Medium.	Percentage of germination.
Distilled water	60
Soil decoction	95
Soil extract, sterilized	95
Soil extract, unsterilized	10
Onion decoction	99
Onion leaf extract	0
Onion leaf extract, diluted with distilled water 1 to 10	0
Onion scale extract	0
Onion scale extract, diluted with distilled water 1 to 10	0

[1] Onion decoction: 100 gm. onion scale in 500 cc. distilled water steamed one hour, filtered, and sterilized.
[2] Onion leaf extract: Fresh onion leaves (green) crushed and the sap extracted by squeezing through cheesecloth.
[3] Onion scale extract: Fresh onion scale crushed and the sap extracted as in onion leaf extract.
[4] Soil extract: 500 gm. black loam soil was supported in a glass funnel by excelsior and absorbent cotton; 500 cc. of tap water were poured over the soil; the filtrate was collected twice, and each time it was poured over the soil. The third filtrate was divided into two parts; one part was left unsterilized and the other part was sterilized in tubes at 15 pounds pressure for ½ hour.
[5] Soil decoction: 500 gm. of black loam soil, to which had been added 500 cc. of distilled water, was steamed at 15 pounds pressure for ½ hour. The liquid was filtered through filter paper and sterilized in tubes at 15 pounds pressure for ½ hour.

The striking outcome of this comparison is the marked retardation in unsterilized soil extract and the complete inhibition in onion leaf and onion scale extract. Even when the last two were diluted with 10 parts of water no germination occurred. As pointed out in a previous note by the writer (*38*), further experiments have shown the presence of at least two distinct substances in onion tissue which are probably responsible for inhibition of spore germination. A more detailed study of this phase and its relation to the parasitism of the fungus will be included in another paper. Cooked soil extract, soil decoction, and onion decoction stimulate germination and promote rapid growth of the germ tubes. It is evident that the cooking of the onion scale removes or destroys the substances which are unfavorable for spore germination.

RELATION OF TEMPERATURE.—Since conidia were found to germinate well in distilled water, this medium was used for studies of the effect of temperature on spore germination. A large number of tests were run at a gradation of temperatures ranging from 1° to 35° C. Spores were found to germinate between the limits of 4° and 32°. Appressoria developed in germination drops throughout the same range of temperature. At 35° to 37° slight swelling of the spores took place, giving them the appearance of "involution forms," but normal germination did not occur. Figure 5 is a graphic representation of the effect of temperature as indicated by percentage of conidia germinating in distilled water at 12 hours. Best germination occurred at about 20°, but good germination occurred between 13° and 25°.

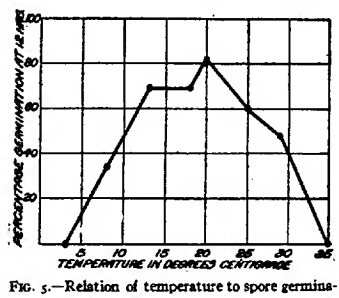

FIG. 5.—Relation of temperature to spore germination of *Colletotrichum circinans*.

The temperature range for spore germination thus coincides closely with that of fungous growth. The point of optimum development is comparatively high, and this fact is significant in exolaining the occurrence of the disease in the field.

EFFECT OF DESICCATION

In order to interpret more fully the development of the disease in the field and the overwintering of the causal organism, the effect of desiccation on conidia and stromata was studied in the laboratory.

ON CONIDIA.—Studies were made on conidia as they occur (1) in masses on the fruiting body on the host, where they are embedded in the mucilaginous material which surrounds them, (2) in similar masses on potato agar, and (3) in water suspension, where the spores are separated from one another, approximating to some extent conditions as

they occur in nature when spores are disseminated by meteoric water. Diseased onions bearing spore masses were brought in and allowed to dry out gradually in the laboratory, and the viability of the spores was tested from time to time. Ordinarily a large percentage lost their vitality within 2 weeks, but in some cases good germination occurred after 7 weeks. A small percentage of conidia from spore masses produced on potato agar and exposed to similar conditions germinated after 4 months. Spores in water suspension allowed to dry out on glass slides were very sensitive to desiccation, little or no germination occurring after 24 hours. It is evident, then, that the conidia are sensitive to desiccation except when they remain in waxy masses on the host, in which condition a small percentage will remain viable through extended unfavorable periods. These results are in accord with the findings of Hasselbring (*14*) for the somewhat closely related fungus *Gloeosporium fructigenum*, causing the bitter-rot of apple.

ON STROMATA.—The stromata of the fungus are capable of withstanding very long periods of desiccation. Test tube cultures of the fungus on a large number of media were kept at room temperature for a period of two years. Since the tubes were not plugged very tightly with cotton the cultures dried out completely within four or five months. The vitality of the fungus in this desiccated condition was tested by adding sterile melted potato agar to the tube and slanting them until the fresh medium hardened. Vigorous growth characteristic of the fungus resulted from the cultures originally made on potato, beef broth, carrot, corn meal, oatmeal, and onion agars, steamed rice and bean pods, and fresh potato and onion plugs. The fungus was no longer viable on synthetic agar, steamed potato, carrot, onion, and fresh carrot. Since spores lose their vitality in such a long period of drying, it may be inferred that the fungus lived through this extended period of desiccation by means of the stromata which developed in the substrate. It is to be expected from these results that the stromata which develop in the scales of the host are capable of carrying the fungus over long periods of unfavorable climatic conditions.

EFFECT OF FREEZING

ON CONIDIA.—Spores in water suspension exposed to freezing temperatures are killed within a few hours. Fresh spore masses also are very sensitive to low temperatures, but if they are allowed to dry out before being exposed to freezing temperatures they will withstand such temperatures for a month or more. In order to test the resistance of conidia to the freezing weather of the entire winter period, infected onion bulbs bearing spore masses were placed out of doors in a weather instrument shelter at Madison, Wis., on December 7, 1915. Germination tests showed a high percentage of these conidia to be viable at this

time. Tests made on January 22, 1916, showed that by this date all the spores had been killed. A similar experiment was carried out at Madison in the winter of 1919–20. Infected bulbs bearing abundance of spore masses were placed out of doors in October, 1919, and protected from rain and snow. A few viable spores were obtained on March 20, 1920. Thus, a few conidia may withstand Wisconsin winters if sufficiently protected, but probably few, if any, live over under field conditions.

ON STROMATA.—Agar cultures containing abundant stromateoid development were kept out of doors during the winter months at Madison, Wis., during which period there was much severely cold weather. In all cases the cultures were found to be viable at the end of this time. Stromata on onion scales have also been exposed in this region during the winter period, and in every case they withstood the severe freezing temperatures.

It is to be expected from the foregoing data that spore masses withstand short intervals of dry weather during the summer and furnish ready inoculum upon the return of moist conditions. During extended periods of unfavorable conditions, however, the stromata serve best to perpetuate the fungus.

PATHOGENICITY

Inoculation experiments were performed on plants at various stages of growth from young seedlings to mature bulbs.

Sterilized greenhouse loam soil was inoculated by spraying with a water suspension of spores at the time of sowing onion seed. Three hundred seeds of White Globe variety were planted in the inoculated soil and the same number in uninoculated soil. Ten days later, as the cotyledons were coming through the soil, the attack of the fungus became evident by the rapid collapse of the succulent tissue at any point on the young shoot. Acervuli of the fungus were present and continued to develop on the diseased portions of the plants. Fifteen days after sowing, 64 out of 123 plants in the inoculated pot were diseased, whereas all of the 161 plants in the control pot were healthy. This experiment was repeated several times, and in each case where sterilized soil was inoculated a high percentage of the seedlings were killed. When unsterilized greenhouse soil was used the injury was greatly reduced, the competition of other soil organisms evidently greatly limiting the activity of the smudge fungus. Moreover, damping off of this sort due to smudge has never been noted in old onion set fields, other factors, such as low temperature at this early part of the season, probably limiting the activity of the fungus.

Leaves of half-grown plants were sprayed with a spore suspension and kept in a moist chamber for 24 to 48 hours. The fungus developed and fruited on the lower leaves, which had reached a stage of "physiological old age," but this never occurred on vigorously growing leaves.

The disease was produced many times by means of artificial inoculation of healthy mature onion bulbs with suspensions of spores from pure cultures, and the fungus was readily reisolated. A summary of these inoculations is given in Table II. In certain cases when bulbs kept in a closed chamber were thus inoculated, the experiment was unsuccessful. It was found in such instances that although the spores were capable of germination in water, they did not germinate in the drops on the bulbs. The inhibitive effect of the volatile oil of onion on spore germination was mentioned earlier by the writer (*38*). An accumulation of this substance when several onion bulbs are placed in the small space in a moist chamber may possibly account for this lack of germination. Further studies on this point will be described in a later paper.

More nearly uniform results were secured when sterilized soil was inoculated by spraying with a spore suspension and healthy bulbs then inserted in this medium for a week or 10 days. The outer scales usually became uniformly infected in 7 or 8 days (see Pl. 81, C). When the bulbs were removed and placed in storage, typical invasion of the underlying scales occurred.

TABLE II.—*Summary of inoculation and greenhouse experiments on onion bulbs*

Type of inoculation.	Inoculation No.	Date of inoculation.	Method of inoculation.	Inoculated.			Controls.	
				Number of onions used.	Percentage infected.	Number of days before first note of disease.	Number of onions used.	Percentage infected
In moist chambers.	1,a	July 24	Spray....	5	100	12	0	0
	1	7	...do.....	1	100	6	1	0
	27	Jan. 8	...do.....	5	100	5	5	0
	29	20	...do.....	5	80	13	5	0
	32	20	...do.....	5	0	.....	5	0
	35	July 20	...do.....	4	100	5	1	0
	44	Nov. 21	...do.....	5	100	6	5	0
	49	Dec. 16	...do.....	5	80	18	5	0
	59	Apr. 12	...do.....	3	0	.....	0	0
	61	26	...do.....	2	100	.....	0	0
In soil.....	3	Aug. 26	.....	10	100	.....	10	0
	23	Dec. 3	.....	5	100	8	5	0
	45	Nov. 30	.....	15	100	8	15	0
	50	Dec. 16	.....	9	100	.....	9	0

In general, then, the fungus assumes the rôle of a weak parasite. Actively growing portions of the plant are not attacked except in young seedlings grown under certain conditions. In the field the fungus is confined to the outer leaves or scales, the cells of which are dead or essentially functionless. As the plant approaches maturity the dry outer scales of the bulb are invaded, but the normal fleshy scales are not affected at this time. A few cases have been noted where the fungus

attacked growing scales which were being parasitized by the smut fungus, *Urocystis cepulae*, but apparently a weakening of the plant is necessary before actual invasion of the growing parts occurs. Following harvest there is a gradual invasion of the dormant cells of the fleshy scales of the bulb as previously described. The progress here is usually slow, but in a moist, warm environment there may be a more rapid invasion, resulting in decay of the resting central bud of the onion set.

RELATION OF THE CAUSAL ORGANISM TO THE HOST TISSUE

METHODS

Onion bulbs from which the thin outer scales had been removed were placed in moist chambers. Inoculum consisting of a suspension of spores from pure culture in sterile distilled water was applied to the uninjured surface of the exposed scales, either in drops by means of a platinum loop or as a spray from an atomizer.

For the study of penetration a razor section was cut tangentially from the surface of the scale directly beneath the infection drop so as

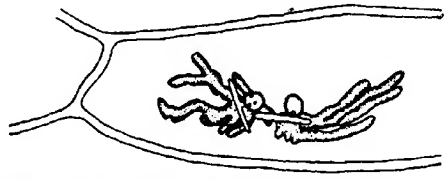

FIG. 6.—*Colletotrichum circinans:* Stage of penetration of epidermal cell of onion scale at 66 hours after inoculation. Camera-lucida sketch. Approximately × 430.

to contain the epidermis with a few layers of the immediately underlying cells. This was examined directly *in toto* in a water mount, the absence of chlorophyll in the host cells making clearing and staining unnecessary. For the study of the relation of the fungus to the host tissue following penetration, pieces of inoculated scale as well as of naturally infected fleshy scales were fixed in Fleming's medium fixative, washed, dehydrated, embedded in paraffin, and sectioned according to standard methods of procedure. In some material a satisfactory differentiation of fungus and host was secured by omitting the bleaching of the microtome sections (commonly done after using a fixative containing osmic acid), which left the mycelium black, and then counterstaining the host cell walls with orange G. In other cases the iron haematoxylin and Delafield's haematoxylin stains gave satisfactory results.

PENETRATION

Under optimum conditions germination occurs within 10 hours and appressoria are formed, either sessile or at the end of short germ tubes. Usually the appressorium is flattened to some extent on the side adja-

cent to the cuticle. The penetration tube is formed from the flattened side of the appressorium and penetrates the cuticle directly (fig. 6, 7). Blackman and Welsford (6) have pointed out that solution of the host cuticle by invading fungi has never been fully demonstrated; they explain the invasion of bean leaf cuticle by *Botrytis cinerea* as mechanical in nature. The mode of penetration in onion smudge was not definitely ascertained, but it seems highly probable that the germ tube from the adhering appressorium might pierce the thin cuticle by means of mechanical pressure.

SUBSEQUENT DEVELOPMENT

The fungus hyphae, after penetration, develop first between the subepidermal wall and the cuticle, which is rather elastic in nature and can be raised considerably without being ruptured. Figure 6 illustrates the extent of invading germ tubes at 66 hours after inoculation. The nature of the penetration tube and the subsequent development beneath the cuticle are shown in figure 7. In certain other anthracnose fungi—namely, *Colletotrichum lagenarium* as reported by Gardner (*12*), *C. lindemuthianum* by Dey (*11*), and *Gloeosporium fructigenum* by Hasselbring (*14*)—the penetration tube has been described as invading the cell wall directly. This is also the case in *Botrytis cinerea* on bean (*6*), although the germ tube in this instance does sometimes grow horizontally beneath the cuticle. The softening of the subcuticular wall in the case of onion smudge soon becomes apparent by its swelling and taking on a laminate appearance. The hyphae grow through and between the laminae (fig. 8) and by rapid development soon form the beginning of the stroma previously described. The swelling of the outer wall eventually involves the entire lumen of the epidermal cell. Although the greatest amount of fungus growth at this stage takes place just beneath the cuticle, occasional hyphae penetrate underlying cells. As the hyphae attack these cell walls, softening and lamination take place as in the subcuticular wall, while penetration is seemingly accomplished partly by means of chemical action and partly by mechanical pressure. The relation of mycelium to the parenchyma cells just beneath the epidermal layer is also shown in figure 8. In the case of bulbs inoculated in moist chambers the collapse of invaded cells was not rapid, and there was no evidence noted of injury to the cells in advance of the mycelium.

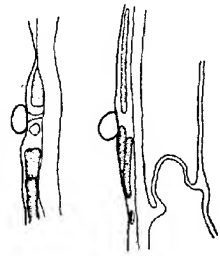

FIG. 7.—Cross section of epidermis, showing early stage of penetration by *Colletotrichum circinans*. Note the empty appressoria with mycelium still wedged between the cuticle and the subcuticular wall. Material fixed 72 hours after inoculation. Camera-lucida sketch. × 700.

Under ordinary storage conditions, the progress of the fungus is closely parallel to that just described, except that the progress is much slower under this different environment. As described before, the first macroscopic symptom of invasion from spots on the dry outer scale to the underlying fleshy scale is a small, yellowish, slightly sunken area. This usually increases in size very slowly in well-ventilated storage. A cross section of one of these spots is illustrated in Plate 83, A, and a detailed drawing from a similar section is shown in figure 9. The fungus develops extensively at first just beneath the cuticle, and the softening and lamination of the subcuticular wall is very slight. As invasion progresses, hyphae penetrate this wall directly, evidently by chemical solution rather than mechanical pressure, since the cavity is slightly larger than the mycelium and there is no sign of bulging of the wall before penetration is achieved. The collapse of cells beneath the epidermal

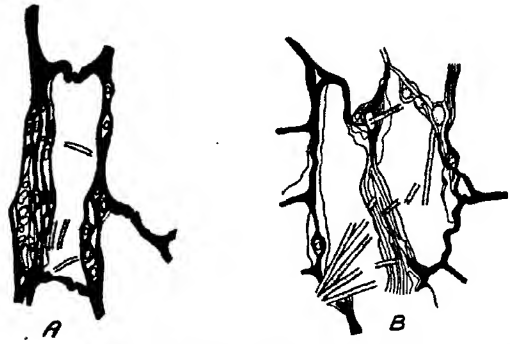

FIG. 8.—Cross section of epidermis (*A*) and underlying parenchyma cells (*B*) of onion scale inoculated with a suspension of *Colletotrichum circinans* spores and kept in a moist chamber at room temperature. Note softening and lamination of cell walls by the invading hyphae. Material fixed five days after inoculation. Camera-lucida sketch. *A*, × 308; *B*, × 350.

cell takes place before any appreciable invasion of hyphae occurs. In the section shown in Plate 83, A, two layers beneath the epidermal layer have collapsed, while only an occasional hypha is to be found beneath the subcuticular wall. There is no evidence of softening of the cell wall. Morever, in such lesions mycelium has never been found in the walls or lumina of turgid living cells. This suggests that either the cells are killed in advance of the hyphae or only slight invasion of the wall leads to their collapse. This slow invasion, which prevails even after the cells have become functionless, is surprising in view of what occurs when bulbs are inoculated in moist chambers. Is it possible that the volatile oil present in the onion scale is influential in checking the advance of the fungus?

Under moist conditions and optimum temperature the stroma develops very rapidly in the subcuticular wall, and acervuli are formed in five to

six days after inoculation. This condition is shown in figure 2. In other cases where sporulation is postponed through lack of proper environment the stroma continues its growth more slowly and eventually involves a larger portion of the scale. The cuticle, however, remains intact on the exterior and normally is not ruptured until the palisade layer of conidiophores is formed. A cross section of a scale which had been held in poorly ventilated storage several months is shown in Plate 83, B. Acervuli were produced upon exposure to proper conditions for sporulation. Note that the cuticle is still present outside the extensive stroma, except where it has been ruptured by the sporiferous hyphae.

FACTORS IN THE PRODUCTION AND PROGRESS OF THE DISEASE

OVERWINTERING OF THE CAUSAL ORGANISM

The experiments already reported on the effect of desiccation and freezing upon conidia indicate only a remote possibility that the fungus lives through the winter in this form under Wisconsin conditions. The stromata, on the other hand, are capable of withstanding protracted periods of drouth or freezing temperature. In order to confirm the supposition that the fungus actually overwinters and is widely disseminated in this latter form, four lots of heavily infected bulbs were placed out of doors at Madison, Wis., on December 7, 1915.. One lot was left in an instrument shelter near the surface of the ground, and the remaining lots were buried in the soil at depths of 2, 4, and 6 inches, respectively. Spore masses were present on this material at the beginning of the experiment, and germination tests showed a high percentage of the conidia to be viable at this time.

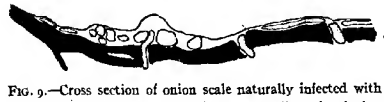

FIG. 9.—Cross section of onion scale naturally infected with *Colletotrichum circinans*, showing the mycelium developing first just beneath the cuticle and later penetrating the subcuticular wall. Camera-lucida sketch. X 450. (This phase is illustrated further in Pl. 83, A).

On January 22, 1916, examination of spores from the bulbs placed in the instrument shelter showed that they had completely lost viability by that date. The four lots of bulbs were examined on April 12, 1916. Those which had been buried in soil readily produced conidia in abundance upon exposure to humid conditions at room temperature. The material kept in the instrument shelter had dried out considerably during the winter and, though much slower to respond, eventually proved to be viable by the production of spores. A similar experiment conducted during the winter of 1916–17 yielded confirmatory data.

It is to be expected that infected scales from the crop of the previous season furnish a source of abundant inoculum for initial infection of the growing crop. This, combined with the fact that in most onion-growing sections it is the common practice to grow this crop successively

on the same field for many years, results in a heavy infection of a large part of the white set crop annually. Examination of a large number of fields in Wisconsin and Illinois has revealed the fact that "clean" white sets are secured as a rule only from land growing its first crop of onions. In a majority of cases the second crop of white sets is badly infected.

In all fields examined where the first crop of onions was being grown, an occasional bulb infected with smudge was found. A satisfactory explanation of these original infections has never been reached. Many possible means of introduction of the fungus from neighboring infected fields immediately suggest themselves, such as manure, farm implements, man and farm animals, drainage water, and wind, and undoubtedly some of these often do play a part in the distribution of the disease. The possibility of seed as a carrier is also to be considered in this connection. Although smudge has never been found attacking the floral parts of the plant, it is conceivable that those seed umbels which fall over and come in contact with the soil before harvest might become infected or be the means of introducing bits of infected scales to the seed. It should be noted in this regard that the spores of onion smut, a disease which is also confined to the bulb and leaves of the plant and in fact does not attack onion seed plants, have previously been found on onion seed samples (*9*, *18*).

One experiment was performed on the relation of seed to the dissemination of the fungus. Samples of six varieties of seed were sown in pots of sterilized soil in the greenhouse on December 5, 1916. On January 16, 1917, all the seedlings were examined. Fruiting bodies of *Colletotrichum circinans* were found on the outer scales of two seedlings of the White Globe variety and of one seedling of the Queen variety. No other signs of the disease were found. The identity of the fungus was confirmed by isolation of pure cultures and comparison with authentic strains. Two subsequent plantings of the same sample of White Globe seed were made, but no further sign of the disease was found. The small amount of the fungus occurring in this experiment is not surprising, since only a very limited amount of infectious material can be expected to be seed-borne. However, although the evidence at hand indicates that the fungus is carried on seed to some extent, further data are necessary before a final conclusion on this point can be made.

RELATION OF TEMPERATURE TO INFECTION AND TO DEVELOPMENT OF THE DISEASE

Studies of the relation of temperature to the germination of conidia and to their subsequent growth have shown the optimum to be about 20° C. for the former and 26° for the latter. The range in each case, however, is wide. Accordingly a set of experiments was started for the purpose of determining the range and optimum temperature for infection.

Sterilized loam soil in glass or glazed crock jars was inoculated with a water suspension of spores. Healthy white onion sets were then

inserted in the soil; and the jars, each covered with a glass plate, were placed in incubators running at temperatures ranging from 5° to 32°.

In the first experiment 10 onions were placed in each of four jars which were placed in incubators held at 5°, 13° to 14°, 23°, and 28° to 31° C., respectively. The extent of the disease on the various lots at this time is shown in Plate 82. It was apparent that infection took place very slowly at 13° to 14°, while that at 28° to 31° was slightly less advanced than at 23°.

In the second experiment jars containing 10 onions each were held at 5° to 6°, 9° to 10°, 14° to 15°, 17° to 18°, 20° to 21.5°, 22° to 23°, 26° to 27°, and 30° to 32° C. They were allowed to remain for 17 days before examination. At the end of this period, no infection had taken place at 5° to 6°, a very slight infection at 9° to 10°, and as the temperature rose the amount of disease increased up to 26° to 27°, at which point it was greater than in any of the other jars. At 31° to 32° it was slightly less than at 26° to 27°. A third experiment confirmed the results of the first two.

Infection takes place and the disease progresses, then, at or above 10° C., but it is quite evident that for very rapid development a temperature of 20° or above is needed. Since the fungus develops in the soil prior to infection, the range of soil temperature during the growing season is undoubtedly an important factor in determining the severity of the disease.

PRODUCTION AND DISSEMINATION OF CONIDIA

After the appearance of the first stromata on the bulbs, subsequent spread of the disease is effected to a considerable extent by conidia

Sporulation does not take place except under fairly humid conditions. In order to determine the range of temperature at which fructification may occur, infected scales were placed in Petri dishes lined with moistened filter paper and exposed in incubators running at a range of temperatures from 2° to 28° C. Abundant sporulation occurred within 36 hours at 20° to 28°. The process was much retarded at lower temperatures, though a few spores were formed at 2° to 3° after several days.

Under optimum conditions for spore production the conidia accumulate on top of the acervuli, forming gelatinous masses which remain intact among the setae. Exposure of portions of scales bearing fresh spore masses over sterile agar plates has yielded no indication of spore discharge. The mucilaginous material surrounding the spores appears to dissolve partly when a spore mass is placed in water, and the conidia thus become separated.

It is thus to be expected from the nature of the fungus that warm, rainy weather is especially favorable for the development of smudge, since high humidity promotes the production of spores, and meteoric water, especially in the form of spattering rain drops, is important for their dispersion and dissemination.

CORRELATION OF CLIMATIC CONDITIONS WITH THE DEVELOPMENT OF THE DISEASE IN 1915–16

Plots of white onion sets were grown in 1915 and 1916 on land which had previously produced many successive crops of onions and where the smudge organism was known to be present in the soil. Soil temperature records were taken at a depth of 1 to 2 inches during part of the 1915 season and most of the 1916 growing season. The daily mean soil temperatures and rainfall for these seasons are represented in figure

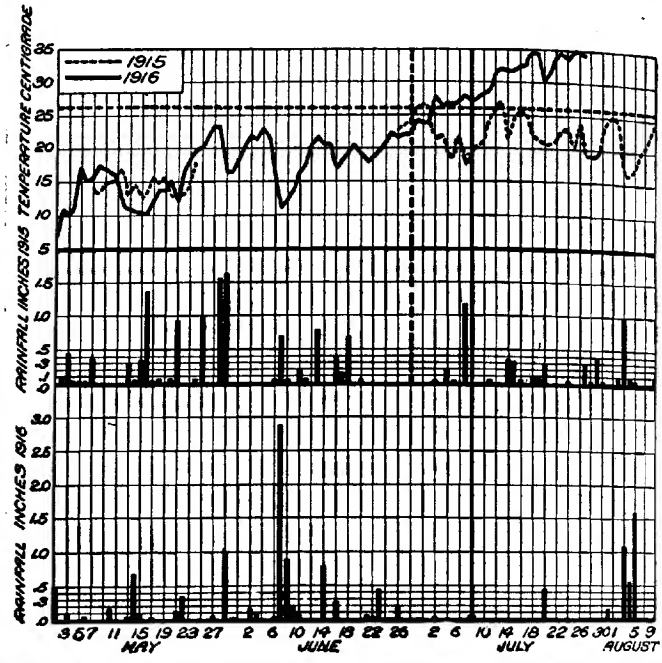

FIG. 10.—Chart from data collected at Racine, Wis., during 1915 and 1916, showing the daily mean soil temperature at a depth of 1 to 2 inches, and the rainfall. The horizontal broken line represents the optimum temperature for infection and development of the disease as indicated by controlled experiments, the broken vertical line the date of first observation of the disease in 1915, and the heavy vertical line the first appearance of the disease in 1916.

10. The rainfall records included here are compiled from data taken at the Racine (Wis.) post office, approximately 3 miles from the onion set plots. The progress of the disease between the time of its first seasonal appearance and harvest is described for these two seasons, since they represent distinctly different conditions which had varying effects upon the progress of the disease.

IN 1915

On June 28 a very few dark green stromata were found, but no acervuli or setae had developed. The soil temperature mean was now well

above 20° C. and remained between 20° and 27° for most of the time until harvest. On July 2 a few scattered acervuli were found. A slight precipitation was recorded on July 2, 2 inches on July 4, 0.02 inch on July 5, and 1.17 inches on July 7. Following this rainy period there was a marked increase in number of acervuli noted on July 10. A slow rain fell during most of July 14 and part of July 15. On July 15 the disease was prevalent above the bulbs on the unthickened portions of the outer leaves which comprise the "neck." These infections were clearly the result of spores spattered upon these portions from the bulb scales by rain a few days previously. The rainy weather, which prevailed until harvest, about August 10, resulted in continued spread and development of the disease, so that the white sets were all badly spotted by the latter date. Further observations showed that the development of the disease in other fields followed closely that noted in the experimental plot. The infection in practically all cases, however, was confined to one or two of the outer dry scales, the fungus being unable to attack the fleshy scales previous to harvest. On the yellow and red varieties the fungus was very abundant on the uncolored portions of the leaves at the neck, but the highly colored bulb scales remained entirely free from it. This has been the usual observation with the colored types.

IN 1916

The month of July, 1916, was extremely warm and dry as contrasted with cool, moist weather of the same period in 1915. The soil temperature mean passed 26° C. on July 2 and remained above that point for the rest of the month. In fact, for a large portion of that period it was well above 32°, the maximum temperature for growth of the fungus on potato agar. No signs of smudge were found until July 8. The extent of the disease at this time was very meager, only a few acervuli being noted. It is probable that the dry weather preceding this date checked the fungus, in spite of the fact that the soil temperature was favorable. Aside from 0.03 inch precipitation on July 8, 0.45 inch on July 20, and 0.14 inch on July 31, no rain fell during the rest of the month. Moreover, the soil temperature was well above the maximum for development of the disease. On July 13 but very little smudge could be found. On July 22 no further development was noted. The moisture from the shower of July 20 disappeared very rapidly from the upper 2 inches of soil because of the extreme heat. A rainy period occurred on August 3, 4, and 5, and following this *Macrosporium porri* and *Phoma alliicola* developed rapidly. Smudge increased but very slowly, however, probably because of the scarcity of viable spores. Another heavy rain fell on August 9 and 10, and the weather then remained clear until after harvest on August 23. At the latter date the bulbs were examined carefully, and in general the sets were only moderately infected. The disease was confined for the most part to the portions of the bulbs below the surface of the soil, while the abundant

infections on the necks which were so conspicuous in 1915 were almost entirely absent.

To summarize, the disease progressed most rapidly during the last part of the growing season of 1915, with the mean temperature range between 20° and 30° C., accompanied by sufficient rainfall to promote abundant spore production and dissemination as well as subsequent infection. On the other hand, development was materially checked in 1916 by extreme heat, together with lack of precipitation during July.

RELATION OF ENVIRONMENT DURING CURING TO THE DISEASE

The onion set crop is usually harvested in early August. The tops are twisted or clipped and the small bulbs are placed in shallow crates 2 or 3 inches deep. These are stacked in the field in piles with temporary roofs, where they are allowed to cure for several weeks. Usually the fungus is well established upon the outer scales of the bulbs before they are pulled, and thus further invasion is dependent largely upon the environmental conditions which prevail during the curing and storage periods.

The respiratory functions of the living cells in the bulbs continue after the sets are pulled, and there is, in consequence, some accumulation of moisture. This is counteracted in part by the use of shallow crates which are exposed to natural air currents. In bright, windy weather the bulbs cure rapidly, while rainy or humid weather retards the process and favors the progress of the disease. A number of experiments were conducted during 1916, 1917, and 1918 to determine the effect of varied amounts of external moisture during the curing period upon the development of the disease.

EXPERIMENT 1.—On August 15, 1916, a crate of white sets was taken from the general run of the crop which had been harvested on August 9 at Racine, Wis. The outer scales were badly spotted with smudge, and in some cases the second scale had been invaded. After removal to the laboratory the bulbs were sprinkled with water while in the crates. After two days a portion of this lot (5½ pounds) was dried for 24 hours at 45° to 52° C. and the remainder (14½ pounds) was given no further treatment. Both lots were placed under cover in a shallow crate, where they were exposed to good conditions for further natural curing. They were later placed in a well-ventilated onion warehouse held at about 35° to 40° F. On January 13, 1917, both lots were examined. Most of the outer dead scales present at harvest time had sloughed off during storage, and in the dried sets the fungus had advanced very little from these original infections. In the naturally cured sets, however, the fungus, probably aided by the greater excess of moisture present, had invaded several underlying scales, and these sets were badly spotted even after the outer scales were removed. The sets in each lot were then sorted into three classes—(1) free from disease, (2) slightly diseased,

(3) badly diseased. The result of this classification is given in Table III, and samples from the dried and the undried lots are shown in Plate 85, A, B.

TABLE III.—*Relation of artificial curing to the development of onion smudge*

Treatment.	Condition at end of storage period.		
	Percentage free from disease.	Percentage slightly diseased.	Percentage badly diseased.
Naturally cured	7	29	64
Artificially dried	56	36	8

EXPERIMENT 2.—On August 30, 1917, several bushels of white onion sets were secured from a field where the crop had been harvested on August 16 and placed in stacks of shallow crates. The weather had been clear during this intervening period, and good natural conditions for curing had prevailed. Smudge was prevalent on the outer scales of the sets at this time. In order to test the effect of exposure to moist weather on the progress of the disease, a portion of this lot in the crates was sprinkled with water daily for one week, approximating roughly what often occurs when a rainy period comes during harvest. After one week a part of the moistened lot was placed in a kiln drier, where the temperature was held at 100° to 120° F., until the bulbs were thoroughly dried. The remainder of this lot was allowed to dry naturally under cover. All the sets were then stored in a standard onion storage house. Samples taken from a moistened and an unmoistened crate on October 10 are shown in Plate 85, C, D. Marked increase in the amount of smudge was very noticeable within a few days after moistening was begun. On January 14, 1917, the amount of smudge was estimated by classifying several hundred bulbs from each of the three lots into either of two classes, namely, (1) those free from smudge or only slightly diseased and (2) those so badly diseased as to impair their market quality. The results are given in Table IV.

TABLE IV.—*Effect of varied conditions at harvest on the amount of smudge on stored onion sets*

Treatment.	Condition at end of storage period.	
	Percentage free from smudge or slightly diseased.	Percentage badly diseased.
Best natural curing	58	42
Exposed to moist conditions after harvest	7	93
Artificially dried after exposure to moist conditions	52	48

This experiment shows (1) that even under what may be considered very good weather conditions for natural curing a considerable amount of smudge will develop; (2) that exposure to moist weather for a week after harvest practically doubled the amount of smudge; and (3) that thorough artificial drying immediately after such exposure counteracts the effect of excessive moisture.

EXPERIMENT 3.—The sets used in this experiment were from a late sowing and consequently were not harvested until September 14, 1918. Smudge was prevalent on the extreme outer scales of a large percentage of the bulbs at this time. Five bushels were placed in shallow crates in the kiln drier, in which the temperature was maintained at 100° to 120° F. One crate was removed at the end of one day, a second at the end of two days, and the remaining three on the fifth day. Three untreated crates used in the experiment were allowed to cure in a covered pile in the field with the remainder of the crop. On September 30 they were removed to a standard onion warehouse, where they were stored during the winter with the artificially dried lots. On March 5, 1919, when final notes were taken, a comparison of the artificially cured and field-cured lots was secured by estimating the percentage showing any signs of smudge after sets had been milled to remove the loose scales.[1] The results are given in Table V.

TABLE V.—*Amount of smudge on artificially cured and field-cured onion sets at the end of the storage period*

Crate No.	Nature of treatment.	Length of treatment.	Percentage showing any signs of smudge.
		Days.	
2	Artificially dried	1	3
3	.....do	3	22
1	.....do	5	21
4	.....do	5	33
5	.....do	5	31
8	Field-cured	16	72
9	.....do	16	75
10	.....do	16	78
	Average of artificially dried crates		22
	Average of field-cured crates		75

The foregoing experiments clearly establish the importance of moisture as a factor in the advance of the disease during the curing and storage periods. They also indicate that artificial curing immediately following harvest greatly checks the progress of the disease as compared with natural field-curing.

[1] It is the common practice to run "bottom" sets through a fanning mill as they are taken from storage in order to remove the loose outer scales.

RELATION OF STORAGE CONDITIONS TO THE DISEASE

The study of the disease in storage has been directed toward the solution of three problems: (1) The importance of smudge as a cause of premature sprouting of sets; (2) the extent of shrinkage, if any, which can be brought about during the storage of onion sets; and (3) the amount of new infection or actual spread from diseased to healthy bulbs occurring during the holding period. While the data on these points are by no means complete and the factors involved in the progress of the disease during the storage period by no means fully studied, the experiments here reported upon throw some light on the matter.

Observations on the first two questions were made in a standard onion set warehouse at Morton Grove, Ill. In practice, onion sets are stored in crates about 4 inches deep with slatted bottoms, piled so as to allow a 1- to 2-inch space between each two crates to facilitate circulation of air. Sets are placed in storage during September and October. The temperature is gradually lowered, following seasonal changes, until it approaches 0° C. (32° F.), an attempt then being made to hold it slightly above this point. During extremely cold weather some artificial heat in the house is necessary to prevent freezing, while ventilation is constantly needed to remove excessive moisture.

The experiments were carried on during the winter of 1918–19. The extremely mild weather during this season prevented the temperature of the house from being held as close to 0° C. as is commonly the case, while, on the other hand, ample opportunity for ventilation was afforded. Continuous records of temperature and relative humidity were secured by means of a Friez hygro-thermograph. The temperature gradually lowered during October and November, the minimum temperature reaching 0.5° C. (33° F.), on November 23, while the maximum temperature commonly reached 12.7° C. (55° F.) during this period. During December, January, and February the temperature fluctuated between 0.5° and 7.2° C. (33° and 45° F.). The relative humidity varied between 65 per cent and 85 per cent during October and November, while throughout the remainder of the period it seldom went above 75 per cent and not often below 60 per cent.

RELATION OF SMUDGE TO SPROUTING

Two lots of onions were used in these experiments, and, since they differed somewhat as to time of maturity and method of handling, they are here considered separately.

EXPERIMENT 1.—Bulbs averaging about 1 inch in diameter were selected from a lot of white sets harvested early in August and brought into storage on August 22, 1918. Two groups were secured, one consisting of 49 bulbs badly spotted with smudge and the other containing 47 perfectly healthy sets. The two lots had thus been grown and handled alike and presumably differed only as to infection with smudge.

They were carried through storage and examined on February 18, 1919. The results are given in Table VI.

TABLE VI.—*Relation of smudge to sprouting of onion sets in storage*

EXPERIMENT 1

Condition of bulbs.	Total number of bulbs used.	Number sprouted.	Percentage sprouted.
Healthy	47	14	29.7
Diseased	49	26	53.0

EXPERIMENT 2.—The sets used in this experiment were sown late in the spring and consequently were not harvested until about September 14, 1918. They were allowed to cure in the field in the normal manner until September 30, when they were placed in storage. Three average crates were selected at this time and kept under observation. At harvest time smudge was prevalent only on the dry outer scales of the sets, but during the storage period it gradually penetrated the underlying scales. When a final examination was made on March 5, 1919, it was clear that in nearly every case where the fungus had penetrated deeply the bulb had sprouted and had thus become worthless. A typical example of this condition is shown in Plate 81, D. An estimate of the amount of sprouting actually due to or intimately associated with smudge was secured by counting 100 to 200 bulbs in each crate. The results are given in Table VII.

TABLE VII.—*Relation of smudge to sprouting of onion sets in storage*

EXPERIMENT 2

Crate No.	Number of bulbs examined.	Total percentage infected by smudge.	Total percentage sprouted.	Total percentage sprouted and showing advanced stage of smudge.
1	165	75	6.0	6.0
2	197	75	9.6	9.6
3	148	72	2.0	.7
Average		74	5.8	5.4

It is not to be construed from these data that smudge is always the chief cause of premature sprouting of onion sets in storage, since unquestionably other factors may often be entirely responsible. One of these, the neckrot decay of the scales, commonly produces a similar effect. It is apparent, however, that the invasion of the bulb scales by the smudge fungus brings about some physiological change which promotes growth of the previously dormant bud.

Economically this factor has considerable value, since bulbs which sprout before the end of the storage period are usually a total loss.

RELATION OF SMUDGE TO SHRINKAGE OF SETS IN STORAGE

In order to secure bulbs as nearly comparable as possible except for presence or absence of smudge, healthy and diseased sets averaging about 1 inch in diameter were selected from a general lot of white sets which had been harvested in early August, properly field-cured, and placed in storage on August 22, 1918. Four lots of 25 bulbs each were secured which showed heavy smudge infection but no signs of any other disease. Three lots of 25 each were selected which appeared to be perfectly healthy. All lots were weighed on October 15. Two diseased lots and one healthy lot were kept in the warehouse throughout the experiment under conditions previously described. In order to secure a high relative humidity a special temporary chamber was made in the warehouse and lined with moistened burlap. Thus, a relative humidity of 90 to 95 per cent was maintained at a temperature close to that of the main warehouse. Two diseased and two healthy lots were placed in this chamber for approximately four weeks and then removed to the main warehouse room. The several lots were weighed on December 30, 1918, and on February 18, 1919. The results of the experiment are given in Table VIII. A constant increase in shrinkage of diseased sets over healthy sets was to be noted. Before the end of the experiment sprouting had occurred in most of the lots, and, as was to be expected, was more prevalent in diseased than in healthy lots. Sprouting and the complication of contaminating parasites should be considered; but, since the former is seemingly enhanced by the disease and the latter is not serious in these cases, there is reason to believe that smudge is responsible in large measure for the increase in shrinkage.

TABLE VIII.—*Relation of smudge to shrinkage of onion sets in storage*

Lot No.	Condition of bulbs.	Environment.	Number of bulbs used.	Original weight, Oct. 15, 1918.	Percentage of shrinkage.		Condition at end of experiment.
					Dec. 30, 1918.	Feb. 18, 1919.	
				Gm.			
1	Diseased	Ordinary storage	25	291.8	6.5	18.7	12 sprouting; 1 infected with neck-rot.
18	do	do	25	277.5	7.4	19.0	15 sprouting.
2	Healthy	do	25	319.3	2.5	11.3	8 sprouting; 1 infected with blue mold.
3	Diseased	Exposure to high relative humidity for 4 weeks, followed by ordinary storage.	25	324.0	8.2	23.3	
20	do	do	25	303.0	8.9	28.8	16 sprouting; 3 infected with neck-rot.
4	Healthy	do	25	324.3	2.8	9.1	7 sprouting.
21	do	do	25	284.5	4.1	11.4	5 sprouting.
Average shrinkage of diseased lots					7.7	22.4	
Average shrinkage of healthy lots					3.1	10.6	

SPREAD OF SMUDGE IN STORAGE

It has been claimed that smudge spreads from infected to healthy bulbs in storage (*17*, *29*). It is to be expected that under unusually moist conditions this might occur. However, since considerable moisture is necessary for sporulation and infection, the conditions which prevail in good storage houses are not conducive to rapid spread of the disease. Several experiments have been conducted during the course of this investigation in which healthy bulbs have been marked and mixed in lots of badly diseased sets. A summary of these experiments appears in Table IX.

TABLE IX.—*Spread of smudge in storage*

Experiment No.	Storage conditions.	Length of experiment.	Number of healthy bulbs used.	Condition at end of experiment.
		Days.		
1	Standard onion warehouse.....	154	34	All healthy.
2	.....do.....	103	40	2 bulbs showed slight infection.
3	Cool cellar.....	66	20	All healthy.
4	.....do.....	208	20	Do.
5	Moist chamber at room temperature.	36	20	6 showed slight infection.

It was found that there was little or no spread of the disease under ordinary storage conditions or in a cool cellar. In a saturated atmosphere some infection of healthy bulbs occurred. In practice, then, some spread from diseased to healthy bulbs is to be expected where sets are exposed to rain or very humid atmosphere such as might occur during the curing period. However, with fairly dry sets kept in cool, well-ventilated storage new infections are probably negligible.

CONTROL OF THE DISEASE

The control of this disease is obviously connected closely with the handling of the crop at or immediately following harvest.

In 1915 a spraying experiment was conducted on a plot of white sets at Racine, Wis. The development of the disease in this plot has been described on pages 708–709. Various schedules were used with 4–4–50 and 8–8–50 Bordeaux mixture plus soap, 4–50 copper sulphate, and 1–10, 1–16, and 1–32 lime sulphur. The sprays were applied upon the bulbs and necks of the plants. Contact with the soil probably reduced the disinfecting property of the chemicals, and their adhesiveness was limited by the nature of the scales and leaves of the onion. No beneficial results were secured even where the first application was made before the first signs of the disease appeared and where the spraying was continued at intervals of three to eight days until harvest. The complete failure of

this experiment was sufficient to show that sprays could not be used successfully for the control of smudge.

Dusting of the sets in the crates at harvest time with lime or sulphur has been suggested by Thaxter (*33*). In 1916 and 1918 dusting experiments with lime, sulphur dust, and dry Bordeaux powder were conducted without any positive results. This is to be expected, since, as a rule, the outer scales of the bulbs became infected before harvest and a disinfectant applied externally could hardly prevent further invasion of underlying scales.

The importance of thorough curing and prevention of exposure to humid conditions after harvest has been emphasized by Thaxter (*33*), Clinton (*10, p. 333*), Massee (*17*), and Stevens and True (*30*). The experiments reported on the effect of drying of bulbs at harvest have shown that rapid dehydration of the outer scales at this time checks further invasion by the fungus to a large degree. Observations in the field by the writer during the years 1914 to 1920 indicate that even the best natural curing weather to be expected in the Middle West is not sufficient to do more than partially check the disease on seriously infected fields.

Artificial curing offers a possible measure of control for smudge, and, as already pointed out (*37*), preliminary experiments indicate that neckrot can also be checked by this treatment. Extensive control experiments carried on in the Chicago district in 1918 have shown that thorough drying very soon after harvest is necessary in order to check smudge materially. In the set-growing district a large portion of the crop is grown on contract to be delivered at a central warehouse as soon as it has cured sufficiently. The expense involved in this treatment would almost necessitate that they be dried at a central point, preferably at the place of storage. Therefore, in order to handle the large quantity received, a fairly rapid process of drying would be essential.

Further experimental work is necessary before artificial drying can be recommended as a general practice, and the results of control experiments are reserved for later publication. In the meantime, the most applicable remedial measures consist in prompt harvest and the best use of natural climatic conditions in curing the white onion set crop, including all possible protection from moist weather. This should be followed by storage in a well-ventilated warehouse held as nearly as possible at 33° to 36° F.

SUMMARY

(1) Smudge is one of the most common diseases of white onion sets in Wisconsin and Illinois.

(2) It occurs also on shallot (*Allium ascalonicum*) and leek (*A. porrum*).

(3) The disease was first described by Berkeley in England in 1851 and is now widely distributed in Europe and America.

(4) Smudge is confined to the scales and neck of the bulb, where it causes dark green to black spots. On fleshy scales it appears as sunken yellowish spots which enlarge slowly, coincident with gradual shrinkage of the scale. On colored varieties the disease is confined to unpigmented portions of the outer scales of the neck of the bulb.

(5) Spots on the outer scales of bulbs due to *Macrosporium porri*, *M. parasiticum*, *Phoma alliicola*, and *Urocystis cepulae* may be confused with smudge.

(6) Smudge becomes detrimental to the onion crop as a cause of (1) the reduction of market value of white varieties, (2) shrinkage in storage, and (3) premature sprouting of sets in storage.

(7) A detailed description of the morphology of the causal organism, *Colletotrichum circinans* (Berk.) Voglino, is given. The ascigerous form, *Cleistothecopsis circinans*, has been described by Stevens and True, but complete proof of its connection with *Colletotrichum circinans* is lacking.

(8) Inasmuch as the causal organism produces a subcuticular stroma and a well-defined acervulus, the species is classified in the Melanconiales as *Colletotrichum circinans* (Berk.) Voglino. A comparative study of the latter with *C. fructus* (S. and H.) Sacc. was made.

(9) The characteristic growth of the organism on culture media is described.

(10) Growth on potato agar takes place between 2° and 32° C., while the optimum is about 26°.

(11) Spore germination is stimulated in soil decoction, onion decoction, and sterilized soil extract, as compared with that in distilled water, while it is reduced in unsterilized soil extract and entirely inhibited in onion leaf or scale extract.

(12) Spore germination occurs within the range of 4° and 32° C., while the optimum temperature is from 20° to 26°.

(13) Conidia are very sensitive to desiccation except when in spore masses, in which condition a small percentage retain vitality for four months or more. Stromata are very resistant to desiccation, retaining vitality for two years or more.

(14) Conidia are sensitive to freezing temperatures, but dried spore masses may withstand this environment for a month or more. Stromata are capable of withstanding several months of freezing weather.

(15) The fungus is pathogenic upon the scales of mature bulbs, but does not attack actively growing parts of the plant with the exception of young seedlings, upon which it may cause "damping off" under certain greenhouse conditions.

(16) Spores germinate and appressoria form within 10 to 12 hours. The infection tube is pushed from the side of the appressorium adjacent to the host cuticle directly through the latter. The mycelium then develops for a time between the cuticle and the subcuticular wall, raising

the former and eventually causing a softening of the latter. In bulbs inoculated in moist chambers the fungus progresses fairly rapidly, causing softening and lamination of the walls and the gradual collapse of the cell. The stroma involves the subcuticular wall at first and later the underlying cells, but the cuticle remains unbroken until the acervulus is formed. The process of invasion under storage conditions is essentially the same but much slower.

(17) The fungus overwinters as stromata in infected scales.

(18) Infection occurs at or above 10° C., but progress is very slow below 20°; the optimum is about 26°.

(19) Conidia are produced abundantly under moist conditions and at temperatures between 20° and 30° C. They are disseminated chiefly by meteoric water, especially spattering rain.

(20) The disease develops most rapidly in the field when the mean soil temperature range lies between 20° and 30° C. and is accompanied by abundant rainfall. Extremely hot, dry weather in July checks progress. Presence of moisture favors the progress of the disease during the curing period, whereas artificial drying of sets immediately following harvest checks it.

(21) Smudge tends to promote premature sprouting and increases shrinkage of sets in storage. The disease may spread from bulb to bulb in the crate under very moist conditions, but in proper storage this factor is negligible.

(22) The important measures of control are protection of the harvested crop from rain, rapid and thorough curing, and provision of well-ventilated storage at about 33° to 36° F.

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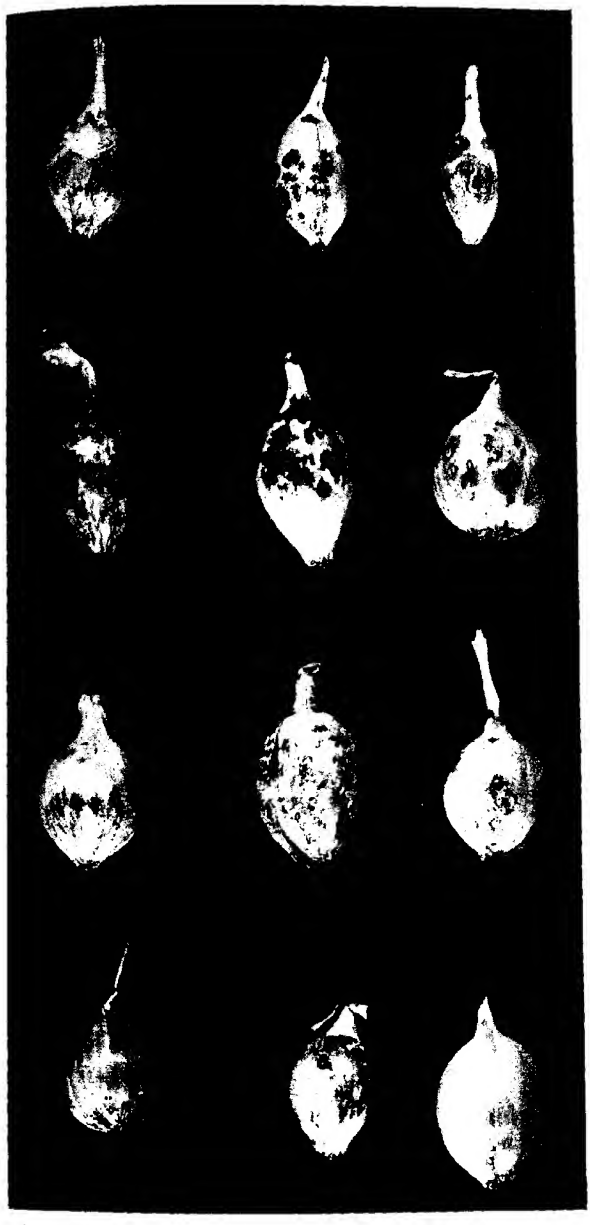
PLATE 80

Onion smudge:

Onion sets (White Portugal variety) naturally infected with *Colletotrichum circinans*. Collected on August 27, 1919, several weeks after harvest, at Morton Grove, Ill. Photographed September 23, 1919. Note in the three lower bulbs the small sunken spots in the fleshy scales which mark the early stages of invasion of the living tissue. Natural size.



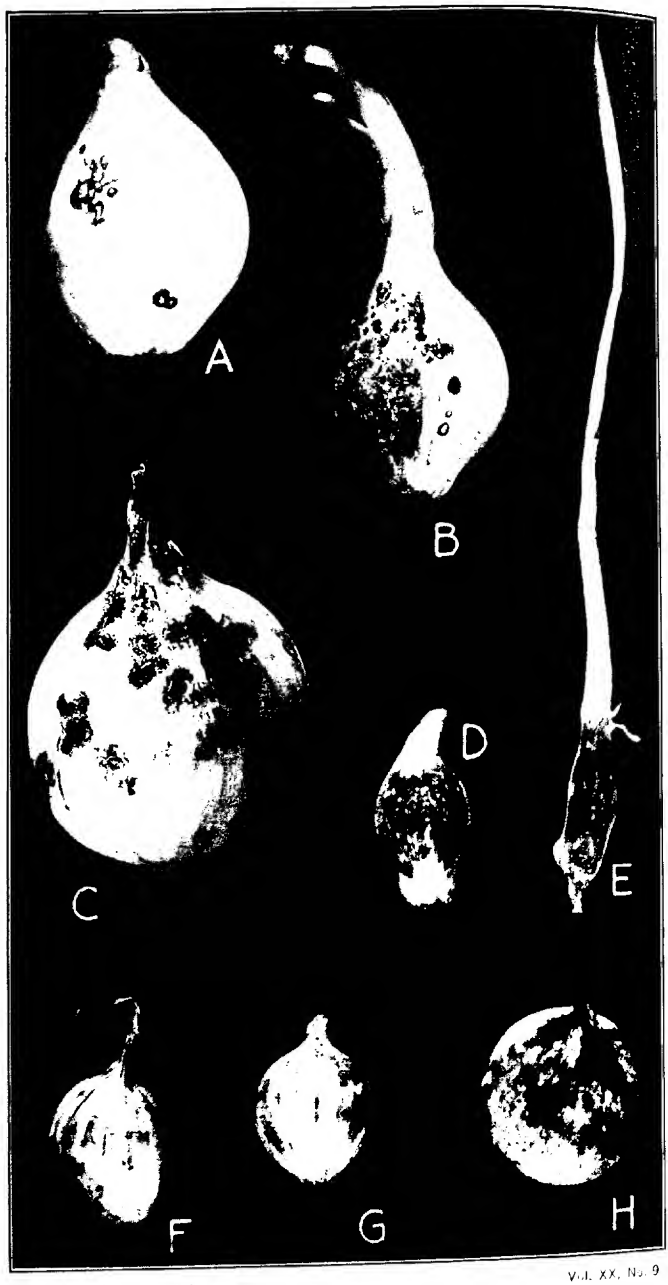

A
B
C
D
E
F
G
H

PLATE 81

Onion smudge:

A, B, E, D.—Advanced stages of smudge after several months in storage. Note the shrinkage of fleshy scales and the tendency to sprout.

C.—Bulb inoculated in a moist chamber with a suspension of *Colletotrichum circinans* conidia.

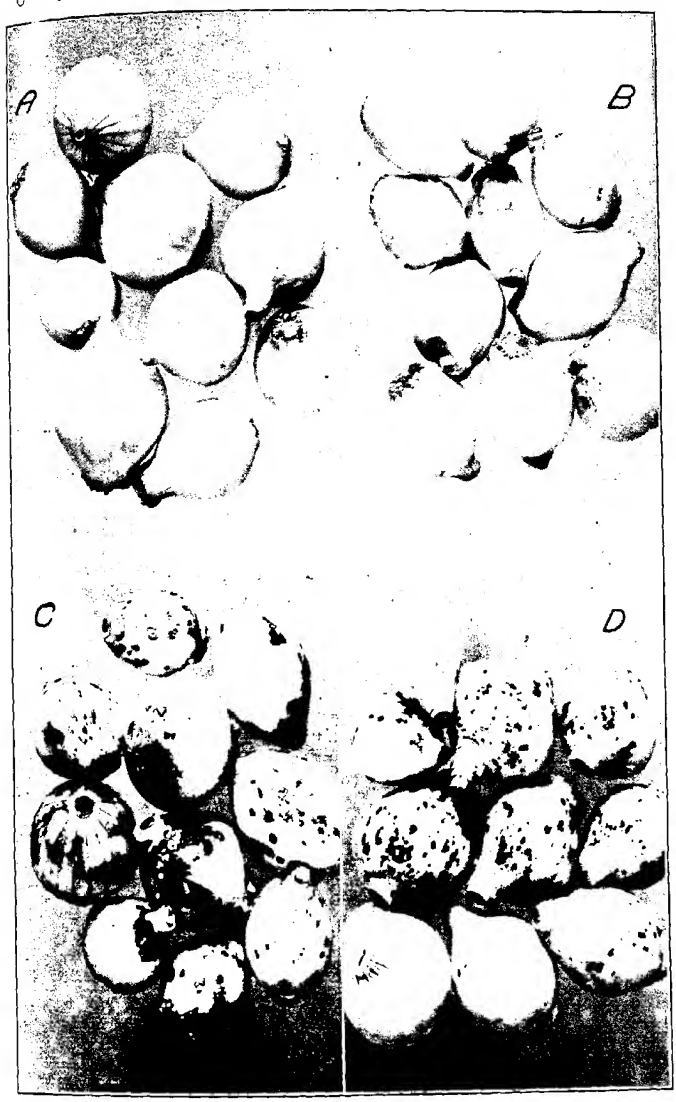
F, G.—*Macrosporium* sp. on outer scale of white onion sets.

H.—*M. porri* and *Phoma alliicola* on outer scale of white onion set. Natural size.

PLATE 82

Relation of soil temperature to the development of smudge:

Onions kept in infected soil held at different temperature for nine days.
A.—5° C.
B.—15° C.
C.—23° C.
D.—32° C.
Slightly reduced.


A
B
C
D

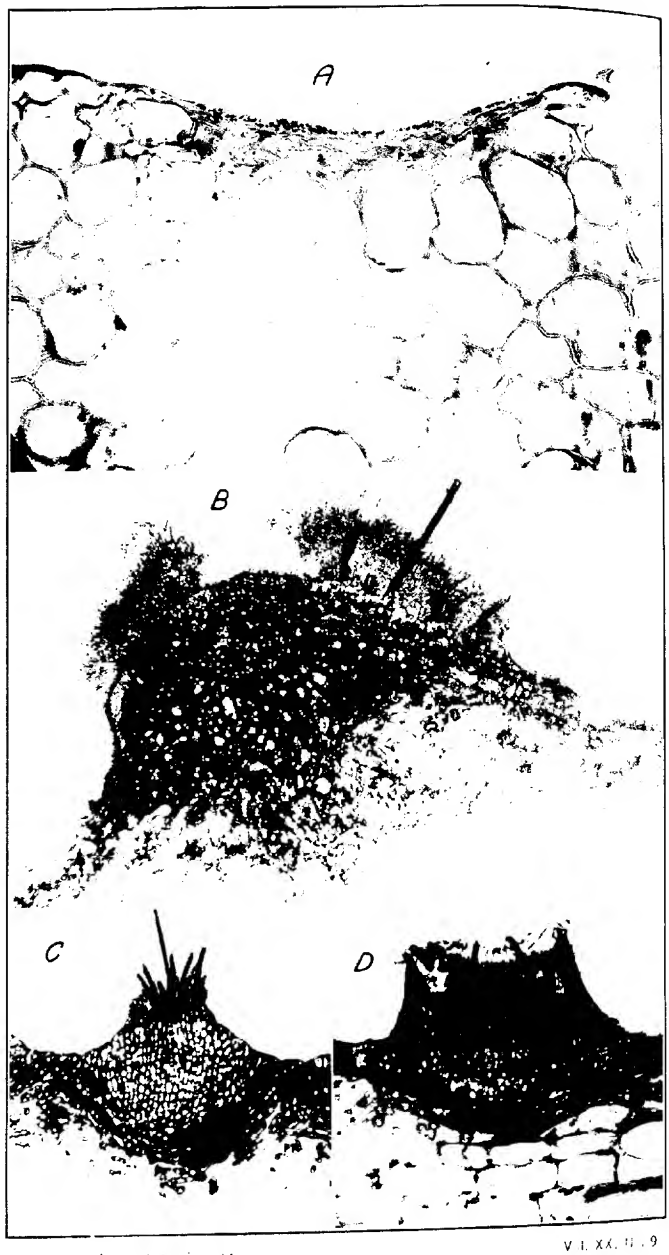

A
B
C
D

PLATE 83

Colletotrichum circinans and *C. fructus:*

A.—Photomicrograph of cross section of naturally infected onion scale. Note that the fungus is confined largely between the cuticle and the subcuticular wall. The epidermal cells and two layers of the parenchyma cells have collapsed, while the uninvaded cells beneath the lesion are slightly enlarged and distended.

B.—Photomicrograph of cross section of an infected onion scale held for several months in poorly ventilated storage. Note that the stroma is excessively developed and that the cuticle is still intact except where ruptured by the acervuli.

C, D.—Photomicrographs of cross sections of *C. circinans* (C) and *C. fructus* (D) on apple fruit. Note similarity between the two forms and the subcuticular origin of the stromata in each case.

PLATE 84

Colletotrichum fructus and *C. circinans:*

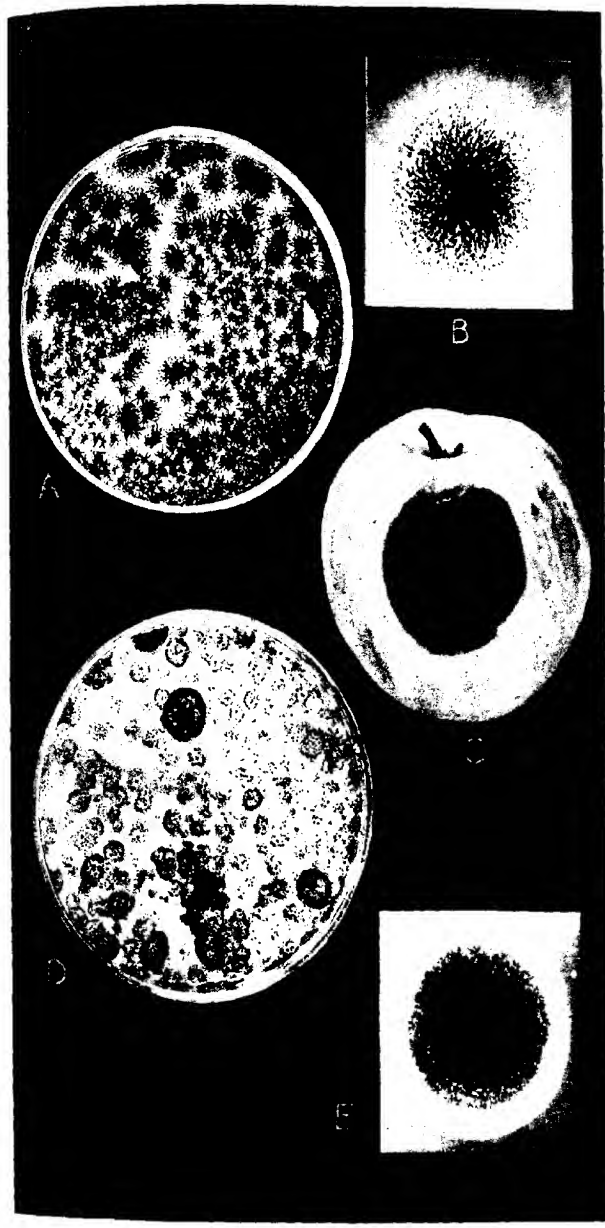
A.—Dilution plate from spores of *Colletotrichum fructus*. Photographed on sixth day. Note stellate character of colonies as compared with *C. circinans* in D. × 4/5.

B.—Individual colony of *C. fructus* on potato agar. Photographed on the fourth day. Compare with *C. circinans* in E. × 1¾.

C.—Apple of Fameuse variety inoculated with mycelium from pure culture of *C. circinans*. Photographed two months after inoculation.

D.—Dilution plate from spores of *C. circinans*. Photographed on sixth day. Compare with *C. fructus* in A. × 4/5.

E.—Individual colony of *C. circinans* on potato agar. Photographed on fourth day. Compare with *C. fructus* in B. × 1¾.


A
B
C
D
E

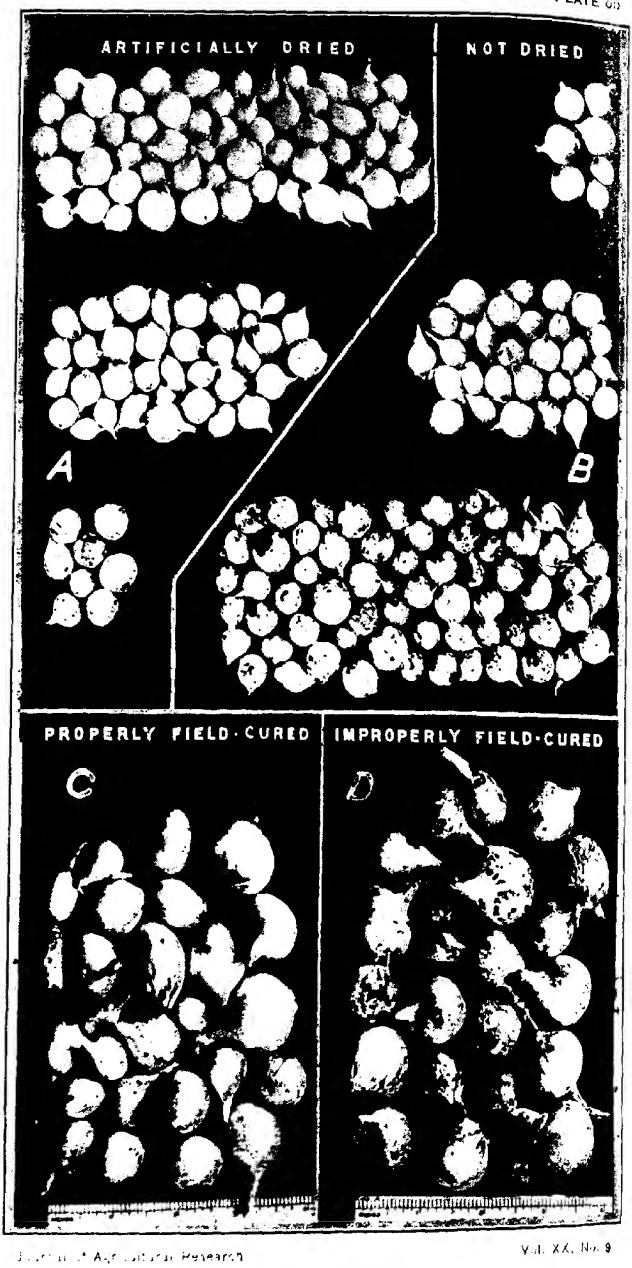

ARTIFICIALLY DRIED
NOT DRIED
A
B
PROPERLY FIELD-CURED
IMPROPERLY FIELD-CURED
C
D

PLATE 85

Relation of curing conditions to the development of smudge:

A, B.—Comparison of onion sets artificially dried immediately after harvest with those not dried. Photograph made at the end of the storage period after the two lots had each been divided into three classes—namely, those free from disease, those slightly diseased, and those badly diseased. (See experiment 1, p. 710–711.)

C, D.—Comparison of white onion sets cured in shallow crates in the field under the best of natural conditions with part of the same lot after exposure to moist conditions for one week. (See experiment 2, p. 711–712.)

25119°—21——5

VARIATIONS IN COLLETOTRICHUM GLOEOSPORIOIDES[1]

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The diseases of citrus trees and fruit known as wither-tip, leafspot, anthracnose, and tearstain are all caused by the same fungus, *Colletotrichum gloeosporioides* (Penz.). These diseases have been found in Florida, (*4; 5; 9, p. 88*),[3] California (*3*), West Indies, South America, Australia, and Malta; and in practically all citrus-growing regions rather serious outbreaks of some or all of these diseases have occurred from time to time.

The smaller twigs of citrus trees are very frequently and severely attacked by the fungus. It is quite common to see many of the small twigs killed back 4 or 5 inches. These infected twigs soon turn to a light brown color and sooner or later become dotted over with numerous small black acervuli. After the rainy season begins, the spores, which are imbedded in a gelatinous matrix, exude from the acervuli and are washed down over the fruit and leaves, causing leafspot, tearstain, and anthracnose of the fruit.

The spores must have an abundance of moisture in order to germinate. Since the rainy season in California occurs during the winter and early spring months, it is at this period that these diseases are most prevalent. In Florida these diseases cause much damage to the citrus industry, whereas in California they are considered of minor importance. This difference in the amount of injury in the two States named is due, I believe, to the difference in the amount of rainfall. During the dry summer in California there is little evidence that *Colletotrichum gloeosporioides* is active. In Florida this fungus causes bloom drop and a considerable amount of leaf spotting during the spring and summer months, as well as anthracnose and tearstaining of the ripe fruit. Many growers and agricultural workers believe that the fungus injury is secondary. It has been stated repeatedly that the weak or injured tree is more susceptible to an attack of *C. gloeosporioides* than the healthy tree.

DESCRIPTION AND HISTORY OF THE FUNGUS

The fungus, *Colletotrichum gloeosporioides* (Penz.) was first described by Penzig in 1882 as *Vermicularia glocosporioides*. In 1887 he placed

[1] Paper No. 66, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Resigned June 1, 1918.

[3] Reference is made by number (italic) to "Literature cited," p. 735-736.

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it in the genus Colletotrichum. It was first collected in America in 1886 by Dr. Martin from Green Cove Springs, Fla., and was first reported by L. M. Underwood (*8*) in 1891. However, the disease was not found in California until some years later. It was reported by Essig (*4*) in 1909 from the Limoneira Ranch at Santa Paula, where it was causing considerable damage to lemon trees.

In 1904, Prof. P. H. Rolfs (*5*) gave a very good description of the fungus as it occurred on various citrus trees and fruits in Florida. He says (*5, p. 20*) that the—

> diseases . . . manifest themselves as wither-tip on orange, pomelo, and lemon twigs; as leaf-spot on the leaves of the various citrous species; as anthracnose on lime blossoms, recently set limes, lime twigs, and lemon twigs; as lemon-spot on ripe lemons and as canker of limes.

The following description is given by Prof. P. H. Rolfs:

> Acervuli located on the surface of the leaf, twig or fruit; 90–270 μ in diameter, erumpent, superficial. Shape various, not uniform, occurring on either surface of citrus leaves; disposed irregularly or in more or less concentric lines; pale to dark colored. On tender lime twigs, tender lemon twigs, lemon fruits and lime fruits, pale colored, dull red in masses, confluent. Epidermis breaks irregularly. Setae fuligi-nous, ranging in length from 60–160 μ, frequently once or twice septate, disposed at margin of acervuli. Frequently absent, and on tender lime twigs, tender lemon twigs, lemon fruits and lime fruits usually absent.
>
> Conidia broadly oval or oblong, 10–16 μ by 5–7 μ, hyaline; size variable in same acervulus, usually with one or two oil drops. Developing from a well-defined stroma; basidia, 3–18 μ. In moist chambers the conidia stream from the break in the epi-dermis. Intrabasidial setae, variable 8–30μ by 3–6μ, cylindrical or sometimes en-larged at distal end; hyaline.

In 1912 Clausen (*1*) described the fungus causing wither-tip of the lime, *Citrus medica*, as *Gloeosporium limetticolum*. He believes that Rolfs had confused two forms and described them as one. Clausen uses the absence of setae as a distinguishing character from *Colletotrichum gloeosporioides*. It is the opinion of Stoneman (*7*), Edgerton (*2*), and Shear and Wood (*6*) that the setae are variable as to presence or absence and that they are not reliable morphological characters to use in separating genera. I have found them in some of my cultures of *Colletotrichum gloeosporioides*, while in other cultures they were absent. Another character he uses is the lack of a coarsely granular plasma filling the spores. I have found several strains of this fungus which are considered to be *Colletotrichum gloeosporioides*, whose spores are not filled with a coarse granular plasma but appear at first to be homogeneous. Clausen also uses growth characteristics as a means to identify the two strains. Some of my strains had the same growth characteristics as the strain which was obtained from Clausen—that is, a white mycelium and abundant spore production.

Shear and Wood (*6*) in their bulletin on the genus Glomerella, have brought together strains from various hosts and included them in one

species, *Glomerella cingulata*. To my knowledge, the perfect stage of Clausen's fungus has not been found. Several of my strains produced the perfect stage when first isolated, and the spores and asci were the same as described for *G. cingulata*. It is, therefore, the opinion of the writer, which will be presented in the following pages, that *Colletotrichum gloeosporioides* as found in California is a polymorphic species, composed of many strains.

STRAINS IN COLLETOTRICHUM GLOEOSPORIOIDES

In the fall of 1916 when the writer began work at the Citrus Experiment Station, the wish was expressed that he should study *Colletotrichum gloeosporioides*. The different members of the Division of Plant Pathology had isolated several cultures of this fungus from different citrus hosts. Some of these differed from each other in their cultural characteristics. It was suggested that these forms might have different regional distribution, or that their differences might be due to the host. Other isolations were made from the various citrus hosts; and these, together with the cultures obtained from the different members of the Division of Plant Pathology, were given laboratory numbers and were always spoken of as strains. In all, 46 cultures were used in the study. Forty-two of these represented all the important citrus districts of southern California, and there was one each from Texas, Florida, Alabama and one kindly furnished by Dr. C. L. Shear.

CULTURAL CHARACTERISTICS

The various strains were grown on five different media—corn meal agar, green bean plugs, potato agar, lactose-beef agar and oatmeal agar. Each strain was grown on these five different media for a period of 18 months. Transfers were made about every 5 weeks, and a record was kept of the variations in growth occurring in each strain on the various media. While most of the strains exhibited different cultural characteristics on the various media, there were a few whose macroscopic characteristics of the mycelium were much the same on all the media. Not only did each strain vary in its growth characters on the different media but some of the strains differed characteristically from each other. Therefore, the variations exhibited by the various strains in their cultural characteristics made it possible to classify them into the following five groups.

Group I: Mycelium white; spores abundant, salmon-colored in mass.

Group II: Mycelium grey to greenish black on the various media, very little aerial growth on oat agar; spores abundant, salmon-colored or yellowish in mass.

Group III: Mycelium gray to black on various media; no spore masses on oat agar.

Group IV: Mycelium gray to black; spore production so abundant on all media that the surface of the medium is nearly covered by a bacteria-like mass of spores.

Group V: Mycelium gray to black, rather fluffy; no pink spore masses on any medium; spore production scant and on some media no spores produced.

Since the cultural characteristics of some strains changed, it became necessary to reclassify the different strains on the following dates: January 27, 1917; April 16, 1917; September 13, 1917; and February 28, 1918. Very few of the strains remained in the group in which they were placed at the first classification. Under artificial cultivation the characteristics of the various strains changed; therefore, they were placed in different groups (see Table I). There were only three strains whose characteristics remained constant in group I. In group II there was only one strain which remained constant. It will be noticed that in group IV cultures 296 and 299 remained constant until September 13, 1917. At the next date of classification these two strains were placed in group II. No strains were placed in group V until September 13, 1917. This may be due to the fact that under artificial conditions these strains lost their power to produce spores.

TABLE I.—*Classification of strains of Colletotrichum gloeosporioides into groups*

Group No.	Jan. 27, 1917.			Apr. 16, 1917.			Sept. 13, 1917.			Feb. 28, 1918.		
I	[a] 295			[a] 295			[a] 295	[a] 429	934	[a] 295		
	[a] 298			[a] 298			[a] 298	496	955	[a] 298		
	323			323			323	502		[a] 429		
	[a] 429			[a] 429			326	901		496		
										561C		
II	326	475	[a] 990	326	560	[a] 990	325	[a] 990		296	527	926
	459	483		459	561		475			299	536	[a] 990
	496	651		475	651		483			323	536A	
	502	901		483	912		510			325	536B	
	507	912		502	926		536			326	561B	
	510	926		507	934		612			502	620	
	943	940		536	943		615			510	901	
III	297	934		297	496	955	507	912		406	536C	912
	325	955		325	510		560	943		475	560	943
	406			406	910		561			507	561	955
	467			467	940		651			527A	561A	
											527C	612
IV	296			296			296					
	299			299			299					
V							297			297	934	
							467			483	940	
							495			495		
							527			527B		
							940			651		

[a] Culture remained in its original class throughout the work.

VARIATIONS IN SPORE LENGTH

Since such great differences were found in cultural characteristics between the strains, the question arose whether differences could be found in the spore length of the various strains. One hundred spores were measured from each strain. The measurements were made in the fol-

lowing manner: A dilute suspension of the spores taken from green bean plugs was made in sterilized tap water, and a drop of the suspension was placed on a microscope slide and covered with a cover glass. It was necessary to make the measurements quickly, because the spores did not remain quiet for any length of time. The image of the spore was thrown on drawing paper by means of the camera lucida, and the length and width were quickly marked with a pencil. The microscope was so adjusted that 1 micron on the micrometer scale in the eyepiece was equal to 1 millimeter on the paper. Therefore, after the length and width were indicated on the paper the spore size could be quickly ascertained by means of a millimeter rule.

TABLE II.—*Variation in spore length in the different strains of Colletotrichum gloeosporioides*

Strain No.	Number of spores measuring (in microns)—																			
	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
296	1	1	3	9	19	28	18	14	6	....	....	....	....	....	....	....	....	....	....	....
901	....	....	....	....	....	....	....	....	1	1	7	10	12	21	19	16	10	1	1	1
459	....	....	....	....	....	4	5	26	33	27	4	1	....	....	....	....	....	....	....	....
429	....	....	....	1	1	2	10	29	31	18	5	2	0	1	....	....	....	....	....	....
406	....	....	....	1	0	2	4	4	13	21	27	11	12	1	2	2	....	....	....	....
326	....	....	....	....	....	....	3	4	15	25	28	15	8	0	1	....	....	....	....	....
955	....	....	....	1	5	23	17	29	21	2	0	1	....	....	....	....	....	....	....	....
295	....	....	....	1	4	14	16	20	28	8	7	2	....	....	....	....	....	....	....	....
990	1	0	0	0	1	1	2	8	35	25	19	5	3	....	....	....	....	....	....	....
297	....	....	....	....	....	4	4	25	27	23	14	1	2	....	....	....	....	....	....	....
651	....	....	....	....	1	1	0	1	8	16	33	21	12	4	3	....	....	....	....	....
299	....	....	....	....	....	15	16	20	32	13	3	0	1	....	....	....	....	....	....	....
323	....	....	....	....	6	24	33	23	11	1	2	....	....	....	....	....	....	....	....	....
510	....	....	....	....	....	3	4	15	41	19	9	7	1	1	....	....	....	....	....	....
507	....	....	....	....	....	7	22	27	37	6	1	....	....	....	....	....	....	....	....	....
502	....	....	....	....	....	13	32	38	13	2	1	0	1	....	....	....	....	....	....	....
943	....	....	....	....	....	4	6	17	26	18	16	11	2	....	....	....	....	....	....	....
940	....	....	....	....	....	....	6	25	37	21	8	2	1	....	....	....	....	....	....	....
934	....	....	....	2	0	5	10	18	32	16	16	0	1	....	....	....	....	....	....	....
527	....	....	5	16	21	22	11	8	5	3	5	4	....	....	....	....	....	....	....	....
560	....	....	....	....	....	11	16	23	28	13	2	3	3	0	1	....	....	....	....	....
536	....	....	....	....	....	1	2	9	21	30	20	12	4	0	1	....	....	....	....	....
561	....	....	....	....	....	2	3	12	36	20	20	4	1	2	....	....	....	....	....	....
524	....	....	....	....	....	....	2	5	22	39	20	10	2	....	....	....	....	....	....	....
514	....	....	....	....	....	2	1	9	20	33	19	11	5	....	....	....	....	....	....	....
513	....	....	....	....	....	....	1	8	24	43	13	8	3	....	....	....	....	....	....	....
517	....	....	....	....	....	1	1	8	19	44	18	8	1	....	....	....	....	....	....	....
515	....	....	....	....	....	....	1	2	8	21	28	22	13	3	2	....	....	....	....	....
947	....	....	....	....	....	....	2	3	19	35	27	12	0	0	2	....	....	....	....	....
467	....	....	....	....	....	1	2	6	22	21	25	13	9	1	....	....	....	....	....	....
512	....	....	....	....	....	....	4	4	20	36	27	5	2	1	0	1	....	....	....	....
475	....	....	....	....	....	1	2	7	22	26	26	12	2	1	1	....	....	....	....	....
926	....	....	....	....	....	....	3	16	31	27	17	5	1	....	....	....	....	....	....	....
483	....	....	....	....	....	2	2	8	26	28	21	10	1	1	0	1	....	....	....	....
325	....	....	1	0	0	7	11	39	30	9	2	0	0	1	....	....	....	....	....	....
298	....	....	....	1	0	5	9	24	31	21	6	3	....	....	....	....	....	....	....	....

It was soon determined that each strain had a certain range of variability in its spore length and width (see Table II). While there were

individual variations exhibited, yet it was soon determined that many of the strains had the same mode for their spore lengths. Therefore, the cultures were classified in regard to the modal length of the spores (see Table III). The strains varied in their modal spore length from 12 to 20 μ. Most of the strains have their mode at 15 μ.

TABLE III.—*Mode of the spore length of different cultures of Colletotrichum gloeosporioides*

Spore No. measuring (in microns)—								
12	13	14	15	16	17	18	19	20
296	323	325	295	475	326	.....	.....	901
527	.....	502	297	483	406	.....	.....	.....
.....	.....	955	298	512	467	.....	.....	.....
.....	.....	.....	299	513	515	.....	.....	.....
.....	.....	.....	429	514	651	.....	.....	.....
.....	.....	.....	459	517	912	.....	.....	.....
.....	.....	.....	507	524	.....	.....	.....	.....
.....	.....	.....	510	536	.....	.....	.....	.....
.....	.....	.....	560	947	.....	.....	.....	.....
.....	.....	.....	561	.....	.....	.....	.....	.....
.....	.....	.....	926	.....	.....	.....	.....	.....
.....	.....	.....	934	.....	.....	.....	.....	.....
.....	.....	.....	940	.....	.....	.....	.....	.....
.....	.....	.....	943	.....	.....	.....	.....	.....
.....	.....	.....	990	.....	.....	.....	.....	.....

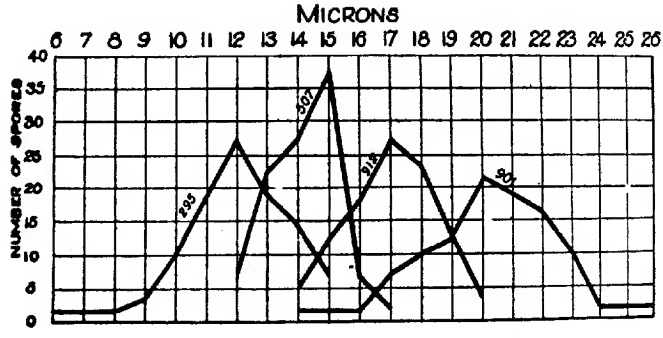

FIG. 1.—Variability of strains of *Colletotrichum gloeosporioides* in spore length.

It was soon observed that this classification could not be correlated with the classification of the strains based on their cultural characteristics. It was hoped that it would be possible to find morphological differences correlated with the cultural characters, but this was not the case.

In order to show the variability within the strain and the differences between the strains, graphs were made representing the variability in four strains (fig. 1). Strain 296 has its modal spore length at 12 μ, 507

has its mode at 15 μ, 912 has its mode at 17 μ, and strain 901, which has the largest spores of all the strains, has its mode at 20 μ.

There was also a certain range of variability in spore width. The variability was not as great as in length. The widths ranged from 3 to 8.5 μ; in most of the strains the mode was about 4 or 5 μ. In strain 901 the variability was from 5 to 8.5 μ with the mode at 6.5 μ.

In Table IV are given the calculated mean, standard deviation, and probable error of each, for the spore length and width of eight different strains. The measurements were made from spores taken from the green bean plug medium.

TABLE IV.—*Table of calculated spore measurements for certain strains of Colletotrichum gloeosporioides*

Strain No.	Mean length of spore in microns.	σ	Mean width of spore in microns.	σ
295	11. 54±0. 065	0. 97±0. 046	5. 52±0. 057	0. 85±0. 041
296	12. 01± .115	1. 71± .082	4. 2 ± .065	.97± .046
298	14. 79± .094	1. 40± .067	4. 68± .014	.21± .010
429	14. 73± .095	1. 42± .068	3. 26± .077	1. 15± .055
507	14. 16± .079	1. 17± .056	4. 91± .048	.71± .034
651	17. 23± .110	1. 64± .078	4. 52± .035	.52± .025
901	20. 34± .137	2. 04± .097	6. 45± .132	1. 96± .093
912	16. 99± .097	1. 44± .069	4. 7 ± .110	1. 63± .078

This table shows that strains grown on the same medium under like conditions vary greatly in respect to their spore sizes. We can, therefore, safely conclude that there exist individual differences in the various strains in regard to certain morphological characters.

VARIATIONS IN THE DIFFERENT STRAINS INDUCED BY THE MEDIUM

The difference in growth characteristics occurring in the same strain when transferred to the various media was very noticeable. The various strains were grown on the five different media for a period of one year. Transfers were then made from cultures growing on the various media to different plates poured with the same medium. The plates were kept at room temperature, and their growth characteristics were noted. It was soon observed that some strains had been more affected than others by their previous environment. While some of the variations were slight, still it was impossible to account for this variation other than as the effect of the medium.

On October 25, 1917, 20 cc. of potato agar were poured in sterilized Petri dishes and allowed to harden. Transfers were then made from the various strains as follows:

STRAIN 429

Plates 1 to 4 were transfers from mycelium on corn meal agar.
Plates 5 to 8 were transfers from spores on corn meal agar.
Plates 9 to 12 were transfers from mycelium on green bean plugs.
Plates 13 to 16 were transfers from mycelium on glucose-potato agar.
Plates 17 to 20 were transfers from mycelium on lactose-beef agar.
Plates 21 to 24 were transfers from mycelium on oatmeal agar (spores).
Plates 25 to 30 were transfers from mycelium on oatmeal agar (mycelium).
On November 22 the final notes taken on the foregoing cultures were as follows:
Plates 1 to 4. White, woolly fungal growth covering the medium. Plate No. 4 was distinctly zoned; spores in center of culture.
Plates 5 to 8. White, scanty fungal growth, which gave the culture a granular appearance; spores in center of culture.
Plates 9 to 12. White, cottony growth, not zoned, but in two plates there was considerable dark mycelial growth; spores in center of culture.
Plates 13 to 16. Very scanty white mycelial growth; few spores.
Plates 17 to 20. White, cottony growth; no spores.
Plates 21 to 24. A membrane-like growth over the entire surface. Very little aerial growth; few spores.
Plates 25 to 30. White, scanty growth of a granular appearance; zoned.

STRAIN 561

Cultures made on glucose potato agar, December 18, 1917.
Plates 1 to 5 were transfers from corn meal agar.
Plates 6 to 10 were transfers from glucose-potato agar.
Plates 11 to 15 were transfers from oatmeal agar.
The final notes were taken on December 28, 1917.
Plates 1 to 5. There is a gray, woolly aerial mycelium; growth in medium is dark In plate 1 there is a white sector; no aerial growth but abundant spore production.
Plates 6 to 10. The growth is white, apprest, wet-looking; no spores.
Plates 11 to 15. No aerial mycelium, zoned, growth in medium white; good spore production on surface.

STRAIN 560

Cultures were made on Petri dishes, poured with corn meal agar December 5, 1917.
Plates 1 to 3 transferred from corn meal agar tubes.
Plates 4 to 6 transferred from green bean plug.
Plates 7 to 9 transferred from glucose-potato agar.
Plates 10 to 12 transferred from lactose-beef agar.
Plates 13 to 15 transferred from oatmeal agar.
On December 17 the final notes taken on the foregoing cultures were as follows:
Plates 1 to 3. White growth in medium; good spore production.
Plates 7 to 9. White growth in medium; no aerial growth; no spores.
Plates 10 to 12. White, woolly aerial growth; no spores.
Plates 13 to 15. Growth in medium, dark; very scant aerial growth; no spores.

STRAIN 990

On October 16, 1917, corn meal agar plates were inoculated with strain 990, the transfers being made from the various media.
Plates 1 to 4 transferred from corn meal agar tube.
Plates 5 to 8 transferred from green bean plug.
Plates 9 to 12 transferred from glucose-potato agar tube.

Plates 13 to 16 transferred from lactose-beef agar tube.
Plates 17 to 20 transferred from lactose-beef agar tube.
Plates 21 to 24 transferred from oatmeal agar (mycelium).
Plates 25 to 28 transferred from oatmeal agar (spores).
The final notes were taken October 29, 1917.
Plates 1 to 4. Gray, short mycelial growth.
Plates 5 to 8. Gray to black aerial mycelium, but in some spots there were no aerial hyphae, growth confined to the medium; good spore production. The peculiar spots were more or less in sector-like areas. Plate 6 showed definite sectors of black and gray aerial mycelium, and in some sections the growth was confined in the medium.
Plates 9 to 12. Almost all the plates had a good growth of gray aerial mycelium, while in others there appeared sectors where the mycelium was confined in the medium.
Plates 13 to 16. No aerial mycelium, but the growth was confined in the medium, was light-colored, and was producing many spores.
Plates 17 to 20. The aerial growth is gray, woolly; some spores produced.
Plates 21 to 24. Gray felt-like growth covering the medium; no spore production.
Plates 25 to 28. These plates differed from plates 21, 22, 23, and 24 in that some of the plates were zoned and produced more spores.

It is clear that there exist variations in a single strain which can not be accounted for on any other ground than the effect of environment. If, therefore, the differences in environment have caused these variations in one year, there may be a possibility of certain environments causing still greater variations which would be more or less permanent.

EFFECT OF THE MEDIUM ON SPORE SIZE

Spores were also measured from the different media to ascertain whether the spore size had been affected. One hundred spores were measured from five different media, and the mean length, mean breadth, standard deviation, and probable error of the mean were calculated for five strains (see Table V). It will be seen that the various media did affect the spore size, but all strains were not affected alike by the same medium. While it has been definitely shown that there exist different strains in *Colletotrichum gloeosporioides*, it also has been shown that these strains are affected in growth characteristics and morphological characters by the medium.

MUTATIONS

The variations which have been described in this paper occurring in the various strains of *Colletotrichum gloeosporioides* have been shown to be due to environmental factors. Not all the variations, however, which occurred during the progress of the work are thought to be due to the environment. These variations which were thought to be induced by some factor or factors other than the environment are in this paper called mutations. These mutations have kept their peculiar characteristics although grown under the same conditions as the cultures from which they arose.

When the various strains were isolated in the fall of 1916, they were grown in plate cultures to study their growth characteristics. The

cultures in which the mutations occurred had greenish gray, fluffy, aerial growth. None of the cultures showed any variation from the description given in the table. This seems to indicate that the cultures were all pure.

TABLE V.—*Differences in spore size of Colletotrichum gloeosporioides induced by various media*

Kind of medium.	Mean spore length in microns.		Mean spore width in microns.	
STRAIN 295				
Corn meal agar	11. 54 ±0. 065	0. 97±0. 046	5. 52±0. 057	0. 85 ±0. 041
Green bean plug	14. 13 ± .114	1. 69± .081	4. 41± .11	1. 63 ± .078
Potato agar	13. 6 ± .129	1. 92± .092	4. 9 ± .055	.835± .040
Lactose agar	15. 74 ± .319	4. 74± .226	4. 65± .044	.65 ± .031
Oat agar	13. 24 ± .071	1. 06± .051	5. 34± .018	.27 ± .013
STRAIN 296				
Corn meal agar	9. 14 ±0. 111	1. 65±0. 079	5. 48±0. 052	0. 77 ±0. 037
Green bean plug	12. 01 ± .115	1. 71± .082	4. 2 ± .065	.97 ± .046
Potato agar	11. 87 ± .118	1. 75± .083	5. 3 ± .03	.44 ± .021
Lactose agar	13. 53 ± .103	1. 53± .073	4..48± .04	.61 ± .029
Oat agar	11. 98 ± .078	1. 16± .055	5. 23± .127	1. 88 ± .090
STRAIN 298				
Corn meal agar	11. 036±0. 176	2. 61±0. 124	3. 34±0. 056	0. 836±0. 040
Green bean plug	14. 79 ± .094	1. 40± .067	4. 68± .014	.21 ± .010
Potato agar	12. 96 ± .144	2. 14± .102	4. 56± .0188	.28 ± .013
Lactose agar	13. 17 ± .126	1. 88± .090	4. 54± .061	.91 ± .043
Oat agar	12. 98 ± .078	1. 16± .055	5. 56± .064	.95 ± .045
STRAIN 429				
Corn meal agar	13. 03 ±0. 138	2. 05±0. 098	3. 94±0. 036	0. 53 ±0. 025
Green bean plug	14. 75 ± .095	1. 42± .068	3. 26± .077	1. 15 ± .055
Potato agar	13. 07 ± .125	1. 87± .089	3. 99± .122	1. 81 ± .086
Lactose agar	12. 75 ± .101	1. 52± .072	3. 75± .047	.70 ± .033
Oat agar	13. 76 ± .123	1. 81± .086	4. 58± .121	1. 80 ± .086
STRAIN 651				
Corn meal agar	14. 43 ±0. 115	1. 71±0. 082	4. 49±0. 013	0. 19 ±0. 009
Green bean plug	17. 23 ± .110	1. 64± .078	4. 52± .035	.52 ± .025
Potato agar	15. 11 ± .115	1. 7 ± .081	5. 12± .017	.25 ± .012
Lactose agar	15. 67 ± .121	1. 8 ± .086	4. 44± .042	.62 ± .030
Oat agar	15. 06 ± .113	1. 67± .080	5. 38± .028	.42 ± .020

In the fall of 1917, after the strains had been grown on artificial media for a year, they were again grown in plate cultures. In a few of the strains there appeared some mycelial growth which differed in color from

the rest of the growth in that plate. These mutations usually appeared as wedge-shaped or fanlike areas with the point of origin usually at the center of the culture. Sometimes they occurred more toward the periphery of the culture. (Pl. 86, A, B.)

Mutations occurred in the following strains: 943, 297, 615, 495, 940, 510, 561, 536, 527, and 990. These mutations have remained true to the characteristics manifested by the first culture. Figure 2 will serve to illustrate the manner in which the mutations originated. Since these strains were not progenies from a single spore, it was thought that there might be a possibility of having a mixture of strains.

There are several types of these variations. One type had a white, fluffy mycelium. A second type, where the mycelium was confined in the medium, had abundant spore production on the surface. A third type had varying shades of gray mycelium bearing spores. At first these peculiarities in growth were regarded as modifications due to some environmental factor. However, after these variations were transferred to other culture tubes and the resulting cultures always exhibited the same characteristics, they then were considered as mutations. Therefore, single-spored cultures were made from one of the strains.

SINGLE-SPORED ISOLATIONS

On November 14, 1917, single spores were isolated from culture 990. The spores were taken from oatmeal agar, and a suspension was made in sterilized distilled water. A platinum loop was used to transfer a drop of the suspension to a cover glass. Each cover glass was examined with the microscope, and when a drop contained only one spore the cover glass was dropped into a test tube containing potato agar. Three cultures were thus obtained and were designated as 990A, 990B, and 990C. After the spores had germinated and had produced a mycelium, transfers were made to the five media used in culturing the various strains. The growth characteristics of cultures 990A, 990B, and 990C were identical with those of the original culture 990.

On November 26, 1917, transfers were made from culture 990C to potato agar plates. The resulting growth was composed of black and white mycelia, with abundant production of spores in the center of the culture (Pl. 86,C). On December 12, 1917, transfers were made from the white and black mycelia to potato agar plates from the cultures made November 26, 1917. The plates made from the black mycelium became black with some white mycelium. The plates made from the white mycelium were white, but only slight traces of black growth could be detected. All cultures produced abundant spores.

On January 9, 1918, transfers were again made from the two kinds of cultures obtained in transfers of December 12, 1917, with results similar to the transfers of December 12, with the exception that there was

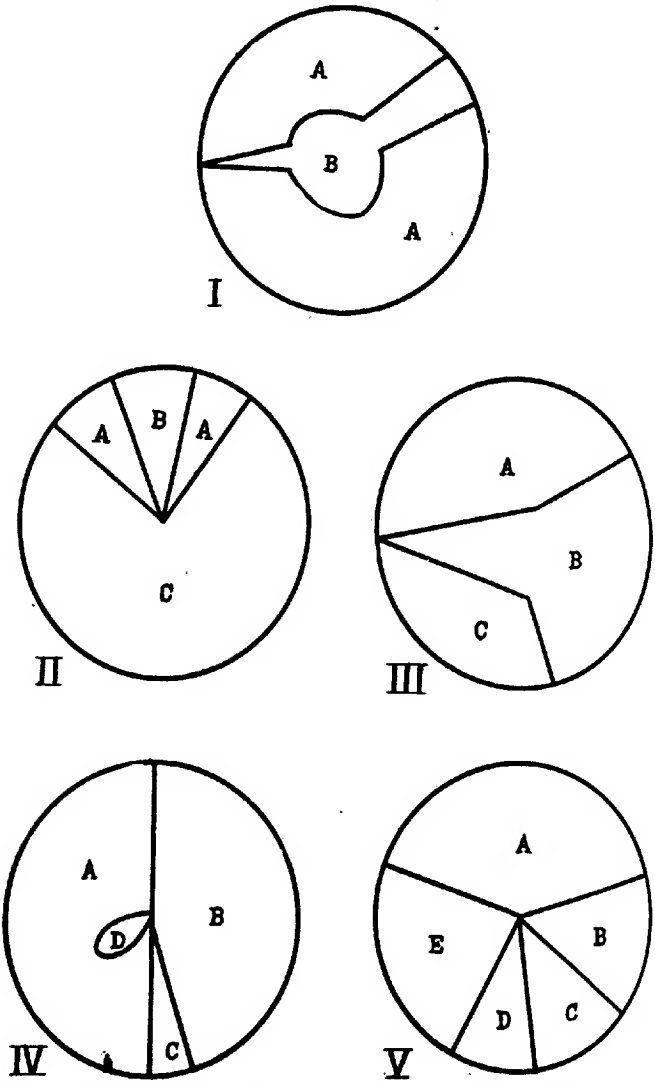

FIG. 2.—I, culture 510: A, greenish black mycelium; B, white mycelium. II, culture 943: A, black mycelium; B, white mycelium; C, mycelium mostly in medium, growth zoned, abundant spore production. III, culture 495: A, black mycelium; B, gray mycelium; C, white mycelium. IV, culture 527: A, gray mycelium; B, greenish black mycelium; C, white mycelium; D, black mycelium. V, culture 940: A, greenish black mycelium; B, white mycelium, some greenish concentric circles; C, black mycelium; D, white mycelium, E, white and black mixed.

practically no black mycelium in the white cultures and but very little white growth in the dark cultures.

Another set of transfers was made on January 24, 1918, from the cultures made January 9, with the result that the white cultures were pure white but the black cultures still produced white hyphae. All plates produced an abundance of spores.

Since the spores are asexual, I wished to determine if they would act like parts of the mycelium when transferred. On January 29, 1918, transfers were made from the spores produced by the white mycelium, and the resulting cultures were pure white, producing many spores. Also spores were transferred from the black and white plates, and the resulting cultures were black with some white hyphae, each culture producing many spores.

The foregoing experiment seems to point to the fact that asexual spores of *Colletotrichum gloeosporioides* act like mycelium when transferred.

The various types obtained by the mutations (fig. 2) are similar to the strains I had in culture. Therefore, one might be led to conclude from the foregoing data that *Colletotrichum gloeosporioides* is constantly giving off new types under natural conditions, as well as in artificial cultures.

SUMMARY

(1) *Colletotrichum gloeosporioides* is a polymorphic species made up of a number of strains.

(2) The various strains when grown on artificial media give distinct cultural characteristics.

(3) Each strain is affected by its environment. The growth characteristics as well as the spore size are varied by the medium on which the strain is grown.

(4) This induced variation may be more or less permanent.

(5) There occur mutations in culture which resemble the strains isolated from the natural environment.

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PLATE 86

A, B.—Variation occurring in strain 990. The cultures were not made from a single spore.

C.—Variation occurring in a culture of strain 990 which was made from a single spore.

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